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PRINCIPAL INVESTIGATOR: Vaughan K. Williams, Ph.D.

CONTRACTING ORGANIZATION: Women's and Children's Hospital
North Adelaide 5006 Australia

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Award

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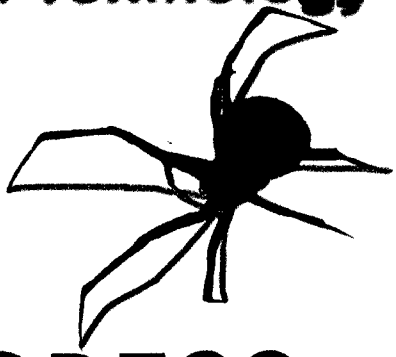
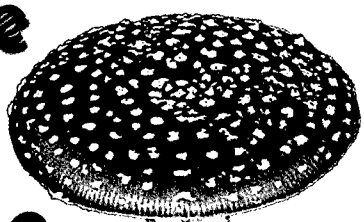


14th WORLD CONGRESS ON ANIMAL, PLANT & MICROBIAL TOXINS

Including Sessions with the
Australian Society of Biophysics
International Society of Thrombosis & Hemostasis

Adelaide Convention Centre
North Terrace, Adelaide
Australia
September 14-19, 2003



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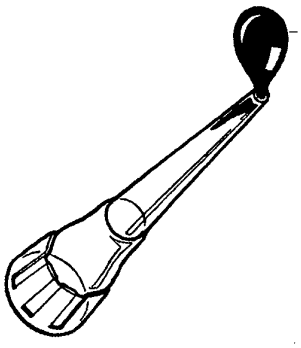

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14th WORLD CONGRESS ON ANIMAL, PLANT AND MICROBIAL TOXINS

Adelaide Convention Centre
Adelaide, Australia

September 14-19, 2003

The Official World Scientific Congress of the International Society on Toxinology

Chairperson of the Organising Committee
Assoc. Prof. Julian White

Co-Chairpersons of the Scientific Committee
Dr. Vaughan Williams
Dr. Richard Lewis

For the International Society on Toxinology
Prof. Dr. Dietrich Mebs

This Congress incorporates a Joint Meeting with the Australian Society of Biophysics

For the ASB
Dr. David Saint

CONGRESS & PROGRAMME BOOK CONTENTS

CONGRESS ORGANISING COMMITTEE

Julian White	Adelaide	Chairperson
Vaughan Williams	Adelaide	Co-chair Scientific Programme
Richard Lewis	Brisbane	Co-chair Scientific Programme
Michael Venning	Adelaide	
Neville Marsh	Adelaide	
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Ray Norton	Melbourne	
Graham Nicholson	Sydney	
Geoff Isbister	Newcastle	
P Gopalakrishnakone	Singapore	
M Kini	Singapore	
Jay Fox	Charlottesville USA	
Len Smith	Frederick USA	
Paul Alewood	Brisbane	
Wayne Hodgson	Melbourne	

CONGRESS PROGRAMME

OUTLINE

Sunday September 14th

Registration available at the South Australian Museum, North Terrace, Adelaide, 1600-1800

Monday September 15th

Adelaide Convention Centre

Congress Registration available from 0800
Congress Official Opening 0845, Hall C
Plenary Lectures 0900-1030, Hall C
Morning Tea 1030-1100
Posters 1100-1140, Glass Foya
Symposia 1140-1300, Hall C, Meeting Rooms 1-3
Lunch 1300-1400, Hall G
Symposia & Oral Papers 1400-1530, Hall C, Meeting Rooms 1-3
Afternoon Tea 1530-1600
Symposia & Oral Papers 1600-1730, Hall C, Meeting Rooms 1-3

Tuesday September 16th

Plenary Lectures 0845-1030, Hall C
Morning Tea 1030-1100
Posters 1100-1140, Glass Foya
Symposia 1140-1300, Hall C, Meeting Rooms 1-3
Lunch 1300-1400, Hall G
Symposia & Oral Papers 1400-1530, Hall C, Meeting Rooms 1-3
Afternoon Tea 1530-1600
Symposia & Oral Papers 1600-1730, Hall C, Meeting Rooms 1-3

Wednesday September 17th

Plenary Lectures 0845-1100, Hall C
Morning Tea 1100-1120
Symposia 1120-1235, Hall C, Meeting Rooms 1-3
Congress Tour 1230-late (includes cut lunch & possibly a simple evening meal)

Thursday September 18th

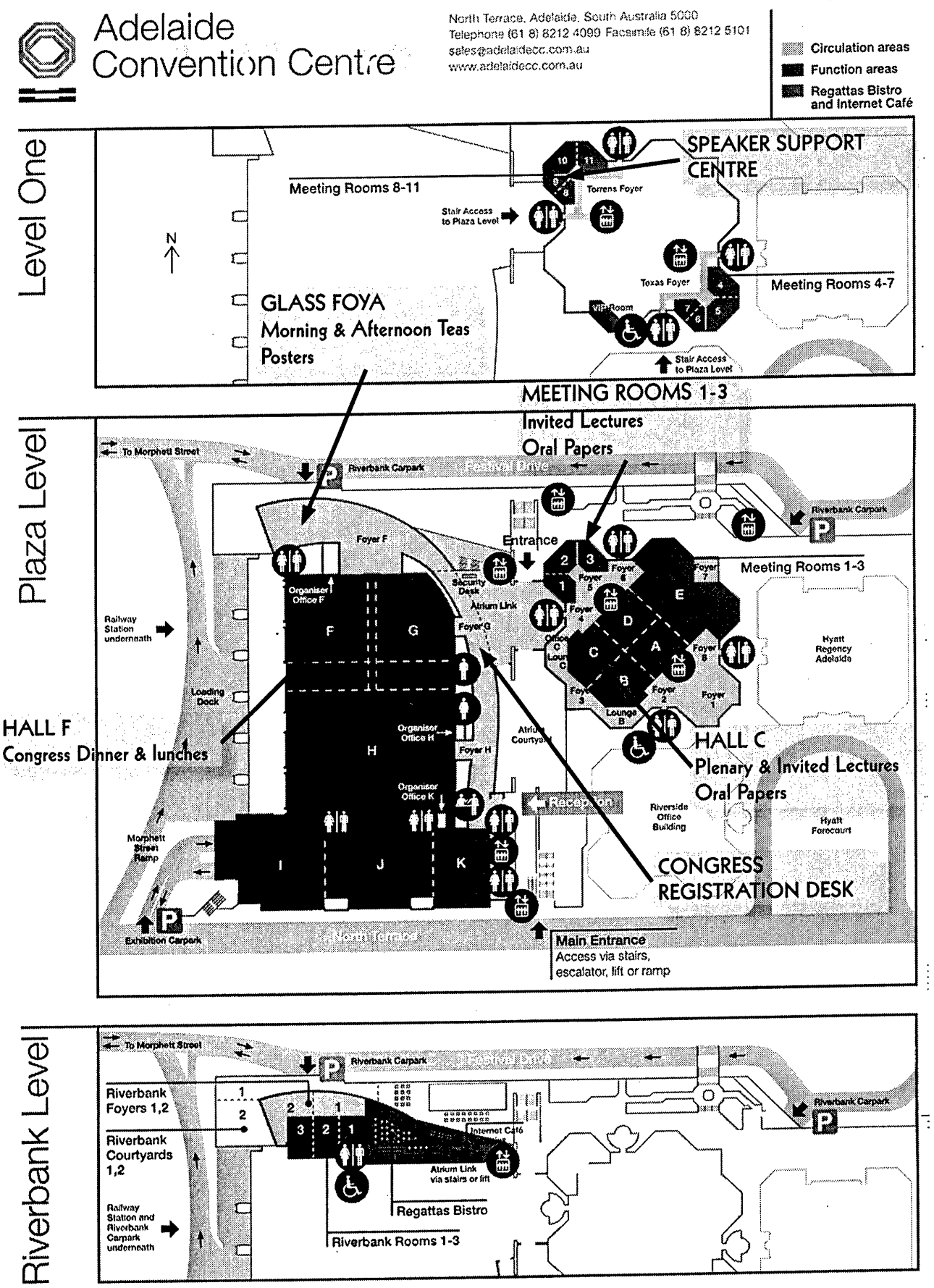
Plenary Lectures 0845-1030, Hall C
Morning Tea 1030-1100
Posters 1100-1140, Glass Foya
Symposia 1140-1300, Hall C, Meeting Rooms 1-3
Lunch 1300-1400, Hall G
Symposia & Oral Papers 1400-1530, Hall C, Meeting Rooms 1-3
Afternoon Tea 1530-1600
Symposia & Oral Papers 1600-1730, Hall C, Meeting Rooms 1-3
Congress Dinner, 1930-late, Convention Centre

Friday September 19th

Redi Lecture & Plenary Lectures 0845-1030, Hall C - Joint meeting with ASB
Morning Tea 1030-1100
Posters 1100-1140, Glass Foya
Symposia 1140-1300, Hall C, Meeting Rooms 1-3
Lunch 1300-1400, Hall G
Symposia & Oral Papers 1400-1530, Hall C, Meeting Rooms 1-3
Afternoon Tea 1530-1600
Symposia & Oral Papers 1600-1700, Hall C, Meeting Rooms 1-3
Congress Close 1700

CONGRESS LOCATION MAP

MAIN VENUE



MONDAY September 15th

ADELAIDE CONVENTION CENTRE, North Terrace, Adelaide

TIME	VENUE & ACTIVITY
0800	Registration desk open
0845-0900	Hall C - Official Opening of Congress
0900-0950	Hall C - Plenary 1AH.1 - Amphibian Toxins - Mike Tyler
0950-1030	Hall C - Plenary 1AH.2 - Aflatoxins - Tom Shier
1030-1100	Foya - Morning Tea
1100-1140	Glass Foya - Poster Session 1PF.1

Session B	Hall C	Meeting Room 1&2
	Structure/Function of Toxins	Biology & Evolution of Toxins
1140-1210	1BH.1 - Paul Alewood	1BM.1 - Mary McLane
1210-1235	1BH.2 - Michael Parker	1BM.2 - Eugene Grishin
1235-1300	1BH.3 - Jan Tytgat	1BM.3 - Dietrich Mebs
1300-1400	Hall G - Lunch	

Session C	Hall C	Meeting Room 1&2	Meeting Room 3
	Structure/Function of Toxins	Biology & Evolution	Clinical Toxinology
1400-1415	1CH.1 - Harris	1CM.1 - Fox	1CR.1 - Marsh
1415-1430	1CH.2 - Tsai	1CM.2 - Ohno	1CR.2 - Warrell
1430-1445	1CH.3 - Pachiappan	1CM.3 - Stenson	1CR.3 - Muller
1445-1500	1CH.4 - Gowda	1CM.4 - Sollod	1CR.4 - Winkel
1500-1515	1CH.5 - Perales	1CM.5 - Nentwig	1CR.5 - Le
1515-1530	1CH.6 - Rehana	1CM.6 - Lumsden	1CR.6 - Bon
1530-1600	Foya - Afternoon tea		

Session D	Hall C	Meeting Room 1&2	Meeting Room 3
	Structure/Function of Toxins	Mycotoxins/Haemostasis	Clinical Toxinology
1600-1615	1DH.1 - Moura da Silva	1DM.1 - Poli	1DR.1 - D'Suze
1615-1630	1DH.2 - Bosmans	1DM.2 - Turk	1DR.2 - Possani
1630-1645	1DH.3 - Dileep	1DM.3 - Schlosberg	1DR.3 - Modler
1645-1700	1DH.4 - Siigur	1DM.4 - Suput	1DR.4 - Sevcik
1700-1715	1DH.5 - Gawade	1DM.5 - Morita	1DR.5 - Williams
1715-1730	1DH.6 - Martin-Eauclaire	1DM.6 - Oda-Ueda	1DR.6 - Viera
1730	Close		

TUESDAY September 16th

ADELAIDE CONVENTION CENTRE, North Terrace, Adelaide

TIME	VENUE & ACTIVITY
0840	Registration desk open
0845-0900	Hall C - Congress announcements
0900-0950	Hall C - Plenary 2AH.1 - Anthrax - Art Friedlander
0950-1030	Hall C - Plenary 2AH.2 - Toxin Induced Inflammation - Jay Fox
1030-1100	Foya - Morning Tea
1100-1140	Glass Foya - Poster Session 2PF.2

Session B	Hall C	Meeting Room 1&2
	Bioterrorism	Toxin Function
1140-1210	2BH.1 - Len Smith	2BM.1 - Ray Norton
1210-1235	2BH.2 - Andrew Robertson	2BM.2 - Ushkaryov
1235-1250	2BH.3 - Gary Phillips	2BM.3 - Moura da Silva (till 1305)
1250-1305	2BH.4 - Gareth Griffiths	
1300-1400	Hall G - Lunch	

Session C	Hall C	Meeting Room 1&2	Meeting Room 3
	Ion Channel Toxins	Bioterrorism	Biology & Evolution
1400-1415	2CH.1 - Kauferstein	2CM.1 - Mark Poli (till 1445)	2CR.1 - Lehvaslaiho
1415-1430	2CH.2 - Corzo	2CM.2 - (Poli contd.)	2CR.2 - Wuster
1430-1445	2CH.3 - Gurevitz	2CM.3 - (Poli contd.)	2CR.3 - Pook
1445-1500	2CH.4 - Wilson	2CM.4 - Ahmed	2CR.4 - Fry
1500-1515	2CH.5 - Gurevitz	2CM.5 - Schlosberg	2CR.5 - Pawlak
1515-1530	2CH.6 - Higuchi	2CM.6 - Meunier	2CR.6 - Bon
1530-1600	Foya - Afternoon tea		
Session D 1600-1730	Hall C - Hypothetical "Game Keeper Turned Poacher - OR - How This Panel Killed This Congress!"		
1830	Close		
1900-late	Toxicon Editorial Panel Dinner		

WEDNESDAY September 17th

ADELAIDE CONVENTION CENTRE, North Terrace, Adelaide

TIME	VENUE & ACTIVITY
0840	Registration desk open
0845-0900	Hall C - Congress announcements
0900-0950	Hall C - Plenary 3AH.1 - Neurotoxins & the ACh Receptor - Titia Sixma
0950-1030	Hall C - Plenary 3AH.2 - Scorpion Toxins - Lourival Possani
1030-1100	Hall C - Plenary 3AH.3 - The Platypus & Toxinology - Roseanne Skalicky
1100-1120	Foya - Morning Tea

Session B	Hall C	Meeting Room 1&2
	Scorpion Toxins	Proteogenomics & Sructure/Function
1120-1145	3BH.1 - Dalia Gordon	3BM.1 - P Gopalakrishnakone
1145-1210	3BH.2 - Michael Gurevitz	3BM.2 - David Craik
1210-1235	3BH.3 - Herve Rochat	3BM.3 - Edward Hawrot
1235	Board buses for Congress Trip - Lunch on trip	

THURSDAY September 18th

ADELAIDE CONVENTION CENTRE, North Terrace, Adelaide

TIME	VENUE & ACTIVITY
0840	Registration desk open
0845-0900	Hall C - Congress announcements
0900-0950	Hall C - Plenary 4AH.1 - Snakebite in Tropical Countries - David Warrell
0950-1030	Hall C - Plenary 4AH.2 - Molecular Basis of Toxic Mini-Proteins - Andre Menez
1030-1100	Foya - Morning Tea
1100-1140	Glass Foya - Poster Session 4PF.3

Session B	Hall C	Meeting Room 1&2
	Understanding Marine Toxins	Haemostasis & Toxins
1140-1210	4BH.1 - Jamie Seymour	4BM.1 - Francis Markland
1210-1235	4BH.2 - David Adams	4BM.2 - Aura Kamiguti
1235-1300	4BH.3 - Richard Lewis	4BM.3 - M Kini
1300-1400	Hall G - Lunch	

Session C	Hall C	Meeting Room 1&2	Meeting Room 3
	Marine Toxinology	Haemostasis	Clinical Toxinology
1400-1415	4CH.1 - Sher	4CM.1 - Serrano	4CR.1 - White
1415-1430	4CH.2 - Nevalainen	4CM.2 - Reza	4CR.2 - Isbister
1430-1445	4CH.3 - Lewis	4CM.3 - St Pierre	4CR.3 - Williams
1445-1500	4CH.4 - Turk	4CM.4 - Banerjee	4CR.4 - Isbister
1500-1515	4CH.5 - Saminathan	4CM.5 - Serrano	4CR.5 - Little
1515-1530	4CH.6 - Bougis	4CM.6 - Bunc	4CR.6 - Alam
1530-1600	Foya - Afternoon tea		

Session D	Hall C	Meeting Room 1&2	Meeting Room 3
	Toxins as Tools	ISTH Cmte Mtg	Clinical Toxinology
1600-1615	4DH.1 - Butera	4DM.1	4DR.1 - Winkel
1615-1630	4DH.2 - Meunier	4DM.2	4DR.2 - Little
1630-1645	4DH.3 - Suput	4DM.3	4DR.3 - Seymour
1645-1700	4DH.4 - Fishman	4DM.4	4DR.4 - Ramasamy
1700-1800	IST Business Meeting - Help decide where the 2006 IST Congress will be held!		
1830	Close		
1900-late	Congress Dinner at Convention Centre		

FRIDAY September 19th

ADELAIDE CONVENTION CENTRE, North Terrace, Adelaide

TIME	VENUE & ACTIVITY
0840	Registration desk open
0845-0900	Hall C - Redi Award Ceremony
0900-0950	Hall C - Redi Award Lecture
0950-1030	Hall C - Plenary 5AH.1 - Shin Ho Chung (ASB)
1030-1100	Foya - Morning Tea
1100-1140	Glass Foya - Poster Session 5PF.4

Session B	Hall C	Meeting Room 1&2
	Clinical Toxinology	Australian Society for Biophysics ASB
1140-1210	5BH.1 - David Theakston	5BM.1 - Dick Wettanhall
1210-1235	5BH.2 - Rick Dart	5BM.2 - Chris Bagely
1235-1300	5BH.3 - Geoff Isbister	5BM.3 - Michael Parker
1300-1400	Hall G - Lunch	

Session C	Hall C	Meeting Room 1&2	Meeting Room 3
	Structure/Function of Toxins	ASB Papers Note: these papers are 20 mins each	Antivenoms
1400-1415	5CH.1 - Serrano	5CM.1 - Martinac	5CR.1 - Theakston
1415-1430	5CH.2 - Ogawa	5CM.2 - Brown	5CR.2 - Warrell
1430-1445	5CH.3 - Spencer	5CM.3 - Barry	5CR.3 - Winkel
1445-1500	5CH.4 - Rossetto	5CM.4 - Rychkov	5CR.4 - Ratanabanangkoon
1500-1515	5CH.5 - Nicholson		5CR.5 - Kiem
1515-1530	5CH.6 - Thwin		5CR.6 - White
1530-1600	Foya - Afternoon tea		

Session D	Hall C	Meeting Room 1&2	Meeting Room 3
	Clinical Round Table	ASB Papers Note: these papers are 20 mins each	Toxins Miscellaneous
1600-1615	5DH.1	5DM.1 - Moens	5DR.1
1615-1630	5DH.2	5DM.2 - Coster	5DR.2
1630-1645	5DH.3	5DM.3 - Gready	5DR.3
1645-1700	5DH.4	5DM.4 - Klonis	5DR.4
1700-1715	5DH.5		5DR.5
1715-1730	5DH.6		5DR.6
1730	Close		

MONDAY September 15th

SCIENTIFIC PROGRAMME

Plenary Session 1AH - Hall C - 0900-1030

Session Chaiperson: Andrez Menez

1AH1 Michael J.Tyler
FROG SKIN GLAND SECRETIONS

1AH2 W. Thomas Shier
APPROACHES TO REDUCING AFLATOXIN CONTAMINATION IN CORN (MAIZE, ZEA MAYS)

Poster Session 1PF.1 - Glass Foya - 1100-1140

Posters for this session listed at end on Monday Abstracts

Invited Lectures Session 1BH - Hall C - 1140-1300 - Structure-Function of Toxins

Session Chairperson: Richard Lewis

1BH1 Paul Alewood
IMPROVING ON NATURE STRUCTURE-ACTIVITY RELATIONSHIPS OF THE TWO-DISULFIDE BOND-CONTAINING CONOTOXINS

1BH2 Michael W Parker
THREE-DIMENSIONAL ATOMIC STRUCTURES OF CHOLESTEROL-DEPENDENT CYTOLYSINS.

1BH3 Jan Tytgat
EVOLUTIONARY ORIGIN OF INHIBITOR CYSTINE KNOT PEPTIDES

Invited Lectures Session 1BM - Meeting Room 1&2 - 1140-1300 - Biology & Evolution of Toxins

Session Chairperson: Herve Rochat

1BM1 Mary Ann McLane
DISINTEGRINS AND INTEGRINS: ALWAYS WORKING TOGETHER?

1BM2 Eugene Grishin
ANALYSIS OF SPIDER VENOM DIVERSITY USING GENOMICS AND PROTEOMICS.

1BM3 Dietrich Mebs
TOXIC SECONDARY METABOLITES: OWN PRODUCTION VERSUS ACQUISITION FROM OTHER SOURCES

Oral Papers Session 1CH - Hall C - 1400-1530 - Structure-Function of Toxins

Session Chairperson: David Craik

1CH1 JB Harris
B-BUNGAROTOXIN AND DEPLETION OF SYNAPTIC VESICLES

1CH2 I. H. Tsai
FUNCTIONAL GENOMICS OF VENOM PHOSPHOLIPASES A2-- OF A PRIMITIVE TREE VIPER TRIMERESURUS PUNICEUS (CROTALINAE)

1CH3 A Pachiappan
TOXICOFUNCTIONAL GENOMICS OF HUMAN BRAIN CELLS EXPOSED TO CANDXOIN, A NOVEL ALPHA NEUROTOXIN OF BUNGARUS CANDIDUS VENOM AND ITS IMPLICATION IN NACHR MEDIATED NEUROTRANSMISSION

1CH4 O Rossetto
STUDY OF THE MECHANISM OF ACTION OF SNAKE PRESYNAPTIC PLA2 NEUROTOXINS

1CH5 Perales, J.
A PROTEOMIC APPROACH TO STUDY THE INTERACTION BETWEEN THE SVMP INHIBITOR DM43 AND DIFFERENT SNAKE VENOMS

1CH6 Syed Rehana
ISOLATION AND CHARACTERIZATION OF A NOVEL PROTEIN FROM THE VENOM OF AUSTRELAPS SUPERBUS

Oral Papers Session 1CM - Meeting Room 1&2 - 1400-1530 - Biology & Evolution of Toxins

Session Chairperson: Dietrich Mebs

1CM1 J Fox
INHIBITION OF SNAKE VENOM METALLOPROTEINASES BY TISSUE INHIBITOR OF METALLOPROTEINASE 3 AND MOLECULAR MODEL OF THE PROTEINAS/INHIBITOR COMPLEX: INSIGHTS INTO THE SIMILARITIES AND DIFFERENCES OF THE REPROLYSINS AND MATRIX METALLOPROTEINASES.

1CM2 M. Ohno
INTERISLAND EVOLUTION OF VENOM PHOSPHOLIPASE A2 ISOZYMES OF TRIMERESURUS FLAVOVIRIDIS SNAKES IN THE SOUTHWESTERN ISLANDS OF JAPAN

1CM3 A.G. Stenson
MOLECULAR EVOLUTION OF PHOSPHOLIPASE A2 GENES IN ASIAN PIT VIPERS.

1CM4 Brianna Sollod
EVOLUTION OF SPIDER TOXIN SUPERFAMILIES AS A STRATEGY TO COMBAT PREY RESISTANCE

1CM5 W Nentwig
SPIDERS OPTIMISE THE USE OF THEIR VENOM

1CM6 Natalie G. Lumsden
THE IN VITRO NEUROTOXICITY OF VENOMS FROM 'HARMLESS' PET SNAKES

Oral Papers Session 1CM - Meeting Room 3 - 1400-1530 - Clinical Toxinology

Session Chairperson: Rick Dart

1CR1 Neville A Marsh
ENVENOMATION BY CAPTIVE SPECIMENS OF THE GABOON VIPER (BITIS GABONICA) – A LESSON TO BE LEARNED

1CR2 D. A. Warrell
LIFE-THREATENING ENVENOMING BY THE SAHARA DESERT HORNED-VIPER (CERASTES CERASTES) CAUSING HAEMOLYTIC URAEMIC SYNDROME: MICRO-ANGIOPATHIC HAEMOLYSIS, COAGULOPATHY AND ACUTE RENAL FAILURE.

1CR3 GJ Müller
THE UNIQUE SYNDROME OF BERG ADDER (BITIS ATROPOS) ENVENOMING

1CR4 Kenneth D Winkel
SNAKE BITE MORTALITY IN PORT MORESBY GENERAL HOSPITAL, PAPUA NEW GUINEA (1992-2001)

1CR5 Le Khac Quyen
CLINICAL EVALUATION OF SNAKEBITE IN VIETNAM: STUDY FROM CHO RAY HOSPITAL

1CR6 Cassian Bon
BIOLOGICAL AND CLINICAL EVALUATION OF ANTIVENOM IMMUNOTHERAPY

Oral Papers Session 1DH - Hall C - 1600-1730 - Structure-Function of Toxins

Session Chairperson: Jan Tytgat

1DH1 Moura-da-Silva, A. M
MOLECULAR CHARACTERIZATION OF TOXINS COMPOSING THALASSOPHRYNE NATTERERI FISH VENOM

1DH2 F Bosmans
IMPORTANCE OF THE CONSERVED AROMATIC RESIDUES IN THE SCORPION ALPHA-LIKE TOXIN BMK M1: THE HYDROPHOBIC SURFACE REGION REVISITED

1DH3 Dileep G.R
EXPRESSION OF OMWAPRIN IN E.COLI, A MEMBER OF WAPRIN FAMILY OF SNAKE VENOM PROTEINS

1DH4 J Siigur
HETERODIMERIC DISINTEGRINS IN VIPERA LEBETINA SNAKE VENOM GLAND ARE SYNTHESIZED FROM DIFFERENT GENES

1DH5 Shivaji P. Gawade
PHOTODYNAMICS OF SNAKE VENOM PROTEINS USING UV SENSITIZED METHYLENE BLUE

1DH6 M-F Martin-Eauclaire
CHARACTERIZATION OF AMM VIII FROM ANDROCTONUS MAURETANICUS MAURETANICUS: A SCORPION TOXIN WHICH DISCRIMINATES BETWEEN NEURONAL AND SKELETAL SODIUM CHANNELS

Oral Papers Session 1DM - Meeting Room 1&2 - 1600-1730 - Mycotoxins/Haemostasis

Session Chairperson: M Kini

1DM1 Mark A Poli
DETECTION OF TOXINS BY ELECTROCHEMICAL LUMINESCENCE

1DM2 T. Turk
OSTREOLYSIN, A CYTOLYTIC PROTEIN FROM THE EDIBLE MUSHROOM PLEUROTUS OSTREATUS AND ITS INTERACTION WITH LIPID MEMBRANES

1DM3 A Shlosberg
DIVERSE TOXICOLOGICAL MANIFESTATIONS DUE TO DIFFERENT SPECTRA OF FUNGAL TOXINS IN 2 MYCOTOXICOSES CAUSED BY ASPERGILLUS SPP. IN ISRAEL

1DM4 D Suput
EFFECTS OF MICROCYSTINS ON RAT AND HUMAN HEPATOCYTES

1DM5 T Morita
CHARACTERIZATION AND CRYSTALLOGRAPHIC STUDIES OF EMS16, AN ANTAGONIST OF COLLAGEN RECEPTOR (GPIA/IIA) FROM THE VENOM OF ECHIS MULTISQUAMATUS

1DM6 N. Oda-Ueda
NOVEL HETEROLOGOUS C3 CONVERTASES FROM TRIMERESURUS FLAVOVIRIDIS VENOM THAT INDEPENDENTLY CLEAVE HUMAN C3AND INITIATE THE COMPLEMENT CASCADE

Oral Papers Session 1DM - Meeting Room 3 - 1600-1730 - Clinical Toxinology

Session Chairperson: Geoff Isbister

1DR1 G. D´Suze1
SUB-CLINICAL ALTERATIONS IN VICTIMS OF SCORPIONISM WITH ONLY LOCAL SYMPTOMS AND THE NEED TO DETECT VENOM QUICKLY.

1DR2 Lourival D. Possani
SCORPION ENVENOMING AND TREATMENT IN MEXICO

1DR3 HT Mödler
CARDIOPULMONARY EFFECTS OF THE VENOM OF PARABUTHUS SCORPION SPECIES IN THE ANAESTHETISED PIG MODEL

1DR4 C. Sevcik
MODELING TITYUS SCORPION VENOM PHARMACOKINETICS: IMPLICATIONS FOR THERAPEUTICS

1DR5 David Williams
PAPUAN TAIPAN (OXYURANUS SCUTELLATUS CANNI) ENVENOMATION IN RURAL PAPUA NEW GUINEA

1DR6 Vieira RJ
DEATH BY BOTHROPIC ACCIDENT. PRESENTATION OF A CASE

MONDAY September 15th

Plenary 1AH.1 - Hall C - 0900-0950

ABSTRACT NUMBER" 05001 1AH.1

FROG SKIN GLAND SECRETIONS

Michael J.Tyler

Department of Environmental Biology, University of Adelaide

Frogs have the most complex skin glands of all vertebrate animals. The glands are classified as mucous, seromucous and granular. The granular glands are commonly referred to as poison glands.

Mucous and seromucous glands are under the control of sympathetic nerves and discharge their secretions upon the surface to reduce body temperature by evaporative cooling.

Granular glands secrete a wide variety of polypeptides and alkaloids with an equally wide range of activities from antibiotic to the most toxic compounds known. Their investigation represents one of the most rewarding fields of natural products pharmacology. Recent findings include the sequestering of terpenes from eucalypts by the local species *Litoria ewingi* and of a plant-derived mosquito repellent by *L. caerulea*. The latter also secretes odiferous bird and rodent repellents. Currently a non-toxic glue is being investigated which has considerable potential in human and veterinary surgery.

Plenary 1AH.2 - Hall C - 0950-1030

ABSTRACT NUMBER" 05701 1AH.2

APPROACHES TO REDUCING AFLATOXIN CONTAMINATION IN CORN (MAIZE, ZEA MAYS)

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Aflatoxins (AF), among the most potent carcinogens known, are produced by some, but not all, strains of *Aspergillus flavus* and *A. parasiticus*. In most countries AF is the most important mycotoxin contaminant of corn, cottonseed, peanuts, tree nuts, copra and other agricultural products. Heat stress is widely accepted to favor AF production. Studies undertaken to better define the conditions which favor AF and fumonisin contamination of corn in Mississippi and Arkansas indicated (i) the most important factor favoring AF contamination was high (>20°C) night temperatures; (ii) corn hybrids selected for AF resistance yielded crops with dramatically lower AF and significantly less fumonisin contamination; and (iii) fumonisin contamination was favored by hot, humid conditions and occurred simultaneously with and independent of AF contamination. Several studies have observed a higher % toxigenic strains in the soil reservoir of *A. flavus* than in naturally-infected crop plants, suggesting that toxigenicity is not a virulence factor. These observations lend support to strategies for reducing AF contamination by preinoculation with non-toxigenic strains. These strategies need a bank of non-toxigenic strains that can be screened for ability to produce stable, infectious spores that compete effectively with toxigenic strains under field conditions. Saito and Machida (*Mycoscience*, 40, 205, 1999) developed a rapid method for identifying AF-producing and non-producing strains of *A. flavus* and *A. parasiticus*, which may provide a useful pre-screen for identifying non-toxigenic strains. In this method, the reverse side of AF-producing colonies turn from yellow to pink immediately after exposure to ammonium hydroxide vapor. The yellow pigments have been isolated and shown to be pH indicator dyes, which turn from yellow to pink at pH ≥8 by addition of any base, and turn yellow again when the pH is lowered with acid. Seven pigments representing most of the color, were identified by comparing spectroscopic and chromatographic properties with literature or predicted values. All are known intermediates on the biosynthetic pathway to AF: versicolorin C, versicolorin A hemiacetal, nidurufin, averufin, versicolorin A, norsolorinic acid, and averantin. Identification of the pigments that predict AF production by *A. flavus* strains as being AF biosynthetic intermediates provides a convenient rationalization for the predictive power of the method.

Invited Lecture 1BH.1 - Hall C - 1140-1210

ABSTRACT NUMBER 05301 1BH1

IMPROVING ON NATURE STRUCTURE-ACTIVITY RELATIONSHIPS OF THE TWO-DISULFIDE BOND-CONTAINING CONOTOXINS

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The Human Genome Project and other major sequencing projects have rapidly provided a vast array of new protein/peptide sequences. In contrast, many other new proteins/peptides are also being uncovered from plant and animal sources whose genomes are yet to be tapped. In the post-genomic era, the physical form of many of these gene-encoded sequences will be vital for biomedical research and drug development. Moreover, the advantages of peptide and protein chemical synthesis over recombinant-DNA methods are increasingly being used to provide rapid structure-activity information of complex bioactive peptides, small proteins and functional receptor domains.

In a program designed to exploit the potential of Australian *Conus* species we have isolated, characterised and chemically synthesised a broad range of novel conotoxins. Subsequently, we have determined their three dimensional structures and in many cases their activities. In general the conotoxins have well-defined tertiary structures with considerable rigidity and stability plus many elements of protein secondary structure present. In this presentation I will describe some of our research on the smaller two-disulfide bond containing conotoxins which suggests that the natural sequences remain amenable to improvement by chemists and in some cases may prove useful in the development of these molecules as therapeutic candidates.

Invited Lecture 1BH.2 - Hall C - 1210-1235

ABSTRACT NUMBER" 13501 1BH.2

THREE-DIMENSIONAL ATOMIC STRUCTURES OF CHOLESTEROL-DEPENDENT CYTOLYSINS.

G Polekhina [1], JJ Adams [1], SC Feil [1], J Rossjohn [1], WJ McKinstry [1], RK Tweten [2], **MW Parker** [1]

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The cholesterol-dependent cytolysins (CDCs) are one of the most widely distributed toxins known, having been identified in five different genera of Gram-positive bacteria. The CDCs exhibit a number of unique features amongst pore-forming toxins including an absolute dependence on the presence of cholesterol-rich membranes for their activity and the formation of very large oligomeric transmembrane pores greater than 15 nm in diameter. There are more than twenty members of the CDC family so far identified and, for those sequenced, there exists a high degree of sequence similarity (40-80% pairwise identities) suggesting they all have similar activities and three-dimensional structures. Here we present crystal structures of three CDC toxins: *Clostridium perfringens* perfringolysin O [1-3], *Streptococcus intermedius* intermedialysin and *Streptoccus pyogenes* streptolysin O. These crystal structures are providing a wealth of information about the mechanism of action of CDCs and possible avenues to treat diseases caused by the bacteria that produce these toxins.

1. Rossjohn, J., Feil, S.C., McKinstry, W.J., Tweten, R.K. and Parker, M.W. (1997) Cell 89, 685-692
2. Shatursky, O., Heuck, A.P., Shepard, L.A., Rossjohn, J., Parker, M.W., Johnson, A.E. and Tweten, R.K. (1999) Cell 99, 293-299.
3. Gilbert, R.J.C., Jiménez, J.L., Chen, S., Tickle, I., Rossjohn, J., Parker, M.W., Andrew, P.W. and Saibil, H. (1999) Cell 97, 647-655.

Invited Lecture 1BH.3 - Hall C - 1235-1300

ABSTRACT NUMBER 11001 1BH3

EVOLUTIONARY ORIGIN OF INHIBITOR CYSTINE KNOT PEPTIDES

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The inhibitor cystine knot (ICK) fold is an evolutionarily conserved structural motif shared by a large group of polypeptides with diverse sequences and bioactivities. Although found in different phyla (animal, plant and fungus), ICK peptides appear to be most prominent in venoms of cone snail and spider. Recently, two scorpion toxins activating a calcium release channel have been found to adopt an ICK fold. We have isolated and identified both cDNA and genomic clones for this family of ICK peptides from the scorpion *Opistophthalmus carinatus*. The gene characterized by three well-delineated exons respectively coding for three structural and functional domains in the toxin precursors illustrates the correlation between exon and module as suggested by 'The Exon Theory of Genes'. Based on the analysis of precursor organisation and gene structure combined with the 3-D fold and functional data, our results highlight a common evolutionary origin for ICK peptides from animals. In contrast, ICK peptides from plant and fungus might be independently evolved from another ancestor.

Invited Lecture 1BM.1 - Meeting Room 1&2 - 1140-1210

ABSTRACT NUMBER 05601 1BM1

DISINTEGRINS AND INTEGRINS: ALWAYS WORKING TOGETHER?

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Disintegrins are small molecular weight, monomeric, homo- or heterodimeric proteins naturally occurring in viper venom, which have been shown to have a remarkable range of potency in interactions with cell surface receptors. Such interactions have been seen with cell types ranging from normal platelets and endothelial cells to various cancer cell lines, including bladder, breast, liver, cervical, fibrosarcoma, lung, neuroglial, ovarian, bone, and melanoma. To date, the cell surface entity involved in this interaction has always been identified as an integrin. Eristostatin, a disintegrin from the viper *Eristocophis macmahoni*, is highly potent and selective for alpha IIb beta 3, with an IC50 of 70 nM in ADP-induced platelet aggregation and a binding affinity (Kd) of 18 nM with either resting or activated platelets. At 10 micrograms/mouse, it inhibits metastasis of human C8161 and M24met melanoma cells in an in vivo model of hematogenous metastasis, while at 25 micrograms/mouse, it also inhibits MV3 human melanoma metastasis. A proposed common mechanism involving an alpha 4 integrin is not likely since the C8161 cell line is lacking this subunit. Analysis of the integrins common to the 3 cells lines shows only alpha 2 and beta 1. Eristostatin has not, however, been shown to interact with either alpha 2 beta 1 or alpha 5 beta 1. A cell surface receptor is a probable candidate since confocal microscopy shows surface labeling on all three melanoma cell lines when using FITC-labeled eristostatin. Crosslinking studies suggest eristostatin is not interacting with either alpha or beta integrin subunits on the cell surfaces of the 3 melanoma cell lines tested. Whatever interactions are occurring are still affected by the presence of an RGD (arginine-glycine-aspartic acid) motif within eristostatin.

Invited Lecture 1BM.1 - Meeting Room 1&2 - 1140-1210

ABSTRACT NUMBER 06701 1BM2

ANALYSIS OF SPIDER VENOM DIVERSITY USING GENOMICS AND PROTEOMICS.

Eugene Grishin[1], Sergey Kozlov[1], Anton Malyavka[1], Bill McCutchen[2], Albert Lu[3], Eric Schepers[3], Rafi Herrmann [3]

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Sequence analysis of the spider toxins available in public domain data bases resulted in formulation of common motifs/patterns present in these toxins. A consensus pattern was developed and used to mine for novel toxins present in 3 species of spiders. cDNA libraries derived from venom glands of *Agelaena orientalis*, *Chiracanthium punctorium* and *Misumena vatia* were constructed and a large number of EST's were sequenced. 5000 sequences were analyzed using the consensus pattern search and toxin diversity in each venom was estimated. Each venom contains about ten different families of toxin-like polypeptides, the number of homologues in each family ranges from 5 up to 25 toxin-like molecules, single forms of polypeptide molecules were also detected. We have identified more than 200 novel putative toxins, many of them belong to unknown structural types of polypeptide toxins.

Invited Lecture 1BM.1 - Meeting Room 1&2 - 1140-1210

ABSTRACT NUMBER 16001 1BM3

TOXIC SECONDARY METABOLITES: OWN PRODUCTION VERSUS ACQUISITION FROM OTHER SOURCES

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In microbes, plants and animals low-molecular toxic compounds are playing an important role in defense such as against predators, in space competition (overgrowth) and infection. Complex metabolic pathways in biosynthesis of these molecules involve numerous enzymes. Modules of enzyme complexes catalyze the synthesis of those toxins, the recombination of these enzymes leads to a wide variety of homologous or even different compounds. De novo biosynthesis, i.e. the own production of secondary toxic metabolites, is an important step in the evolution of toxins, but it is a costly process requiring considerable metabolic energy. Moreover coevolution triggers adaptation and resistance to those toxins, which is observed in many organisms. Toxin tolerance and resistance is also a prerequisite of poisonous and venomous animals to accomodate and survive their own toxicity. On the other hand, numerous animals demonstrate the successful use of external toxin molecules, which they obtain either from their food source, as a product of a mutualistic association with microorganisms or from commensale relationship. Despite the effect of saving metabolic energy, strong dependence on a specific food source or on a particular microorganism is a consequence as well as a risk, particularly when environmental conditions change. This may result in a loss of toxicity. De novo biosynthesis of toxins as well as acquistion of toxicity have their pros and cons. The question, what is cheaper under aspects of saving metabolic energy, is still difficult to answer.

Structure/Function of Toxins - Hall C - 1400-1415

ABSTRACT NUMBER 02001 1CH1

β-BUNGAROTOXIN AND DEPLETION OF SYNAPTIC VESICLES

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We have used quantitative electron microscopy, semi-quantitative fluorescence microscopy and classical physiological techniques to determine the role of voltage gated Ca^{2+} channels, t- and v-snare protein complexes and synaptic vesicle recycling to study synaptic vesicle depletion caused by β-bungarotoxin. Phrenic nerve-hemidiaphragm preparations were maintained in vitro . Mechanical response following indirect stimulation (0.1 H^2) were recorded isometrically. Preparations were exposed to β-bungarotoxin (10μg ml^{-1}). After a lag phase of 10-20 min, mechanical responses began to decline. The responses failed by 210 min. Increasing the rate of stimulation or the dose of β-bungarotoxin slightly reduced the lag phase and time to failure. At the point of failure thin muscle sections were used to assess nerve terminal pathology and frozen sections were used to study nAChE, SNAP-25, syntaxin and synaptophysin distribution. Compared to controls, exposure to β-bungarotoxin caused the destruction of terminal boutons in 10% of end-plates. Where terminal boutons were still intact, synaptic vesicle density was reduced by 80% but terminal bouton area was unaffected. Synaptophysin reactivity was reduced in both area and intensity, but nAChR were unaffected. There was no labelling of SNAP-25 and syntaxin in 10-20% of terminal but labelling was unaffected at the rest. Pre-exposure to botulinum toxin C blocked the depletion of synaptic vesicles and exposure to conotoxin ω MVIIC reduced depletion by 60%. The uptake of FMI-43 into terminals exposed to β-bungarotoxin was unaffected. We conclude that the depletion of synaptic vesicles caused by exposure to β-bungarotoxin depends on the movement of Ca^{2+} through voltage gated Ca^{2+} channels and the formation of t- and v-SNARE complexes. Synaptic vesicle endocytosis is unaffected.

Structure/Function of Toxins - Hall C - 1415-1430

ABSTRACT NUMBER 08201 1CH2

FUNCTIONAL GENOMICS OF VENOM PHOSPHOLIPASES A_2-- OF A PRIMITIVE TREE VIPER TRIMERESURUS PUNICEUS (CROTALINAE)

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By gel filtration and reversed-phase HPLC, a total of six phospholipase A_2 (PLA-) isoforms were purified from individual and pooled venom of the Indonesian brown tree viper *Trimeresurus puniceus*. Their enzymatic activities, N-terminal sequences and precise masses were determined. Using the cDNA prepared from a pair of venom glands of this species, two Lys-49 PLAs, one basic PLA and three acidic Asp-49 PLAs were cloned and sequenced. The molecular masses and the N-terminal sequences of the purified PLAs were matched successfully to each of the cDNA-deduced amino acid sequences. Notably, the basic Lys-49 PLAs of *T. puniceus* contain only six disulfide bridges per molecule and lack the C61-C91 normally conserved in the venom PLAs of pit vipers so far studied. With hardly detectable enzymatic activities, the Lys-49 PLAs induced edema on the rat paws. Their 3-D models are almost identical to the structure of the Lys-49 PLA from *Ce. godmani* venom except the region near position 61. However, they are less stable than a highly similar Lys-49 PLA from *T. stejnegeri* venom as indicated by a decrease of 8.8 °C in melting temperature, when monitored by circular dichroism spectroscopy. On the other hand, a basic anti-coagulating Asp-49 PLA of *T. puniceus* was found to be structurally similar to the basic Gly6Asp49 PLA in the venom of South American Bothrops. This PLA and the three acidic PLAs of *T. puniceus* venom each contains seven pairs of disulfides. The evolution and structural relationships of these PLAs to other pit vipers' PLAs were studied by phylogenetic analyses based on their amino acid sequences.

Structure/Function of Toxins - Hall C - 1430-1445

ABSTRACT NUMBER 17501 1CH3

TOXICOFUNCTIONAL GENOMICS OF HUMAN BRAIN CELLS EXPOSED TO CANDOXIN, A NOVEL ALPHA NEUROTOXIN OF BUNGARUS CANDIDUS VENOM AND ITS IMPLICATION IN NACHR MEDIATED NEUROTRANSMISSION

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Candoxin is a novel three-finger neurotoxin purified from the venom of Malayan krait (Nirthanan et al 2002). It is a reversible antagonist of neuromuscular nAChRs and a poor reversible blocker of neuronal alpha-7 nACh-receptors. Although the biological target of candoxin has been reported earlier, the cellular and molecular mechanism has not been explored. Therefore, the present study explores analysis of differential gene expression profiles at genome level, following exposure of human brain cells to candoxin at different doses (100 and 300nM) and various time points (12, 24 and 48hrs) using Affymetrix Human Gene Chip® (HG-U133A). About 555 genes were differentially expressed (upregulated-305 and downregulated-250) with significantly (≥± 2 fold). Large-scale evaluation of changes in gene expression profiles induced by candoxin implicates its potential role in regulatory (signal transduction- GPCR, MAPK) and metabolic pathways. Other genes involved in multifarious biological process includes, cell communication, inflammatory response, cell growth & maintenance, developmental process, tumor suppressor and apoptosis. Interestingly, our study provides evidence, supporting molecular mechanism of action of candoxin in human brain cells that involves the modulation of genes in regulation of nACh-Receptors and also in peripheral inflammatory lesions. Based on the microarray results, candidate and potential genes were further evaluated and confirmed by RT-PCR. The results suggest that these genes, either individually or grouped in panels play a key role in the regulation of nAChR mediated neurotransmission and signal transduction pathways.

1. Nirthanan S, Charpantier E, Gopalakrishnakone P, Gwee MC, Khoo HE, Cheah LS, Bertrand D, Kini RM. Candoxin, a novel toxin from *Bungarus candidus*, is a reversible antagonist of muscle (alphabetagammadelta) but a poorly reversible antagonist of neuronal alpha-7 nicotinic acetylcholine receptors. (2002) J. Biol. Chem. 277, 17811-17820.

Structure/Function of Toxins - Hall C - 1445-1500

ABSTRACT NUMBER 11701 1CH4

INFLUENCE OF NON-CATALYTIC SITE RESIDUES ON CATALYTIC FUNCTION OF TYPE II PLA_2

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Phospholipase A_2 are esterolytic enzymes that release Sn2 fatty acids from phospholipids. They are 12-14 Kda proteins with a highly conserved catalytic site. The reaction mechanism for all sPLA_2s is a base mediated nucleophilic attack of the ester bond. The roles of various residues of the active site such as Asp 49, 99, His 48, Tyr 52 and 73 have all been very well established. In spite of this, the catalytic activities of different PLA_2s can vary by several orders of magnitude. This observation surely underlines the influence of non-active site amino acid residues on the efficiency of catalytic function. The direct involvement of Asp 49 and His 48 has been clearly established. K49 mutants show little or no enzymatic activity. Similarly, modification of His 48 by different alkylating agents completely abolishes the enzymatic activity. Now, we attempt to investigate the influence of residues other than active site to understand their contribution to enzyme activity. Lysines are generally implicated to be responsible for many of the pharmacological properties of PLA_2. Modification of lysines of a basic PLA_2, VRV-PLV, by KCNO or modification of N-terminal residue by DABITC or removal of N-terminal amino acids by Leucine amino peptidase resulted in the partial loss of enzyme activity. Modification of the only Trp by N-bromosuccinimide gave similar results.

It is a common observation that acidic PLA_2 are highly catalytically active compared to the basic isoforms. A comparison of sequences of acidic and basic PLA_2 shows difference in the distribution of amino acid residues. Only basic or basic rich regions are seen in the N-terminal helix, β-sheet and C-terminal extension of basic Type II PLA_2 while the same regions have more of acidic amino acids in the acidic PLA_2. These could be the regions responsible for the different pharmacological and varying catalytic activities of PLA_2. PLA_2 are assayed at neutral pH at which Histidine's involvement in the nucleophilic attack appears to be most efficient. Also, at this pH acidic residues are negatively charged and basic residues are positively charged. It is possible, the product free fatty acids released may get retarded by the more positively charged residues in basic PLA_2 while the acidic groups in the acidic PLA_2 facilitate their quick release from the enzyme surface. Therefore, the contributions of the charged residues as well as the hydrophobic atmosphere put together add to the catalytic efficiency of the PLA_2s.

Structure/Function of Toxins - Hall C - 1500-1515

ABSTRACT NUMBER 18301 1CH5

A PROTEOMIC APPROACH TO STUDY THE INTERACTION BETWEEN THE SVMP INHIBITOR DM43 AND DIFFERENT SNAKE VENOMS

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The glycoprotein DM43 from opossum serum is an inhibitor of snake venom metalloproteinases (SVMP) whose functional and structural characteristics were recently determined (J. Biol. Chem. 277:13129, 2002). This new member of the immunoglogulin supergene family forms noncovalent complexes with both PI and PIII SVMPs from *Bothrops jararaca* venom. In this study, we have made use of this property to comparatively analyse the metalloproteinase contents of several venoms. DM43 (7 mg) was immobilized on a Hitrap® NHS-activated affinity column (1 mL) through which the following venoms (3 mg each) were separately chromatographed in 0.02 M Tris/HCl pH 7.5 containing 0.02M CaCl2: *Bothrops jararaca* (Bj), *B.insularis* (Bi), *B.atrox* (Bat), *B.asper* (Bas), *Crotalus adamanteus* (Cad), *C.atrox* (Cat), *Lachesis muta muta* (Lm) and *Naja naja atra* (Nn). For each venom, the bound fraction was eluted with 0.1M glycine/HCl pH 2.7 containing 0.02M CaCl2, precipitated with TCA and analysed by 1D SDS-PAGE; the most complex samples were also submitted to 2D SDS-PAGE. The identification of the proteins which interacted with DM43 was done either by N-terminal Edman sequencing or by peptide-mass fingerprinting, using the tool MS-Fit at Protein Prospector WEB Home. According to this methodology, the venoms could be classified into two main groups: Bj, Bi, Bat and Cat venoms presented a more complex profile, with several proteins interacting with DM43, mainly represented by PIII SVMPs; from Bas, Lm and Cad venoms we have isolated mainly PI SVMPs. One faint band with a MW corresponding to a PIII SVMP was isolated from Nn venom. Several isolated proteins could not be identified either as a consequence of N-terminal blockage or due to the absence of structural information about them in the available databanks.

Supported by FAPERJ, PAPES-Fiocruz and CNPq.

Structure/Function of Toxins - Hall C - 1515-1530

ABSTRACT NUMBER 11301 1CH6

ISOLATION AND CHARACTERIZATION OF A NOVEL PROTEIN FROM THE VENOM OF AUSTRELAPS SUPERBUS

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Snake venoms are highly complex mixtures containing many different biologically active proteins and peptides. Australian elapids contain interesting groups of protein toxins distinctly different from other snake venoms, and still remain largely an untapped resource. Recently we purified a protein of molecular mass 8327.38 ± 0.36 from the elapid snake Austrelaps superbus. The sequence obtained after automated Edman degradation showed 100% identity to the C-terminal segment of a chain of CVF from Naja naja. Due to the natural low abundance of this protein (0.5mg/g of crude venom), a synthetic gene was designed, to express it in large quantities in an E.coli expression system. The synthetic gene was cloned in a T7 promoter-controlled plasmid (pET-32a+) suitable for toxic proteins, and expressed as a hybrid protein fused with a N-terminal His-tag to facilitate purification by affinity chromatography. The expression system yielded significant amounts of soluble fusion protein. The recombinant protein was cleaved from the fusion partner and further purified on a RP-HPLC system to carry out the structural and functional characterization of the protein.

Biology & Evolution of Toxins - Meeting Room 1&2 - 1400-1415

ABSTRACT NUMBER 01203 1CM1

INHIBITION OF SNAKE VENOM METALLOPROTEINASES BY TISSUE INHIBITOR OF METALLOPROTEINASE 3 AND MOLECULAR MODEL OF THE PROTEINAS/INHIBITOR COMPLEX: INSIGHTS INTO THE SIMILARITIES AND DIFFERENCES OF THE REPROLYSINS AND MATRIX METALLOPROTEINASES.

Masahide Kashiwagi, Hideaki Nagase, Solange M. T. Serrano, **Jay W. Fox.**

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Snake venom metalloproteinases (SVMPs) and ADAMs belong to the Reprolysin (M12) family of metalloproteinases. In vitro, these proteinases have been demonstrated to be inhibited by α_2 macroglobulin and small peptide inhibitors. Recently, specific members of the ADAMs family, for example ADAM-17 (TNF-α; convertase), ADAM-10, ADAM-TS4 and ADAM-TS5, has been shown to be effectively inhibited by the tissue inhibitor of metalloproteinase 3 (TIMP-3) but significantly less so by TIMPs-1 and -2. Furthermore, early studies from our laboratory with SVMPs and TIMP-1 indicated that this inhibitor was not effective with SVMPs. Given the degree of relatedness of the SVMPs and the ADAMs we re-examined the inhibitory effect of TIMP-1 and -2 on proteolysis of Azocoll by the P-I SVMP, atrolysin c, isolated from the venom of Crotalus atrox. We also assayed for the inhibition of proteolytic activity of atrolysin c by TIMP-3. From these studies we have confirmed the lack of inhibition by TIMPs-1 and -2 on atrolysin c. However, TIMP-3 was a potent inhibitor of atrolysin c with a Ki of approximately 150 nM with a stoichiometry of 1:1. In summary, we have shown that TIMP-3 can effectively inhibit a P-I SVMP which opens the question as to what role endogenous inhibitors such as TIMP-3 may play in envenoming and its subsequent pathological manifestations.

Biology & Evolution of Toxins - Meeting Room 1&2 - 1415-1430

ABSTRACT NUMBER 10001 1CM2

INTERISLAND EVOLUTION OF VENOM PHOSPHOLIPASE A2 ISOZYMES OF TRIMERESURUS FLAVOVIRIDIS SNAKES IN THE SOUTHWESTERN ISLANDS OF JAPAN

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Trimeresurus flavoviridis (Tf) snakes (crotalinae) inhabit the southwestern islands of Japan, namely, Amami-Oshima (A), Tokunoshima (T) and Okinawa (O). Four phospholipase A_2 (PLA_2) isozymes, named PLA_2 ([Asp49]PLA_2), PLA-B (basic [Asp49]PLA_2) and BPI and BPII (both [Lys49]PLA_2), were isolated from Tf (T) venom. BPI and BPII are also present in Tf (A) venom but not in Tf (O) venom. It became evident that in Tf (O) the genes for BPI and BPII are fused to form a pseudogene. Recently we isolated a neurotoxic PLA_2 ([Asp49]PLA_2), named PLA-N, from Tf (A) venom. Its cDNA was also isolated from Tf (T) venom-gland cDNA library. However, Tf (O) venom contains one amino acid-substituted PLA-N homologue, named PLA-N(O). In addition, PLA-B (T), PLA-B' (A) and PL-Y (O), which belong to basic [Asp49]PLA2 category, showed the sequence diversities (one to four amino acid substitutions) in the b-sheet region and its vicinity. These results show that interisland mutation have occurred in the genes encoding such PLA_2 isozymes. Phylogenetic analysis showed that crotalinae snake venom PLA_2s are classified into four PLA_2 groups, PLA_2 type, basic [Asp49]PLA_2 type, [Lys49]PLA_2 type and neuro[Asp49]PLA_2 type. Mathematical analysis indicated that (1) functional diversities were acquired by accelerated evolution and (2) after they acquired particular functions, less frequent mutation has occurred for functional conservation.

Biology & Evolution of Toxins - Meeting Room 1&2 - 1430-1445

ABSTRACT NUMBER 20701 1CM3

MOLECULAR EVOLUTION OF PHOSPHOLIPASE A2 GENES IN ASIAN PIT VIPERS.

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Using primers designed to anneal to the conserved 5' and 3' regions of phospholipase A_2 (PLA_2) genes, mixed locus PCR products have been generated from a range of species from the genus *Trimeresurus* and other genera of Asian pit vipers. By cloning the PCR products, sequences have been obtained from single copies of the gene. In common with previous studies of molecular evolution in this, and other, families of venom genes, a number of general patterns have been observed. Rates of substitution are markedly higher in exons (except for the UTR regions) than in introns, with non-synonymous substitutions equalling or even exceeding synonymous changes. These observations contradict the generally accepted expectations of molecular evolution in protein coding sequences and have caused some workers to seek novel 'molecular mutator mechanisms' to explain the evolution of venom gene families. We outline a mechanism that instead relies on a population genetics phenomenon to explain the observed patterns.

Biology & Evolution of Toxins - Meeting Room 1&2 - 1445-1500

ABSTRACT NUMBER 22801 1CM4

EVOLUTION OF SPIDER TOXIN SUPERFAMILIES AS A STRATEGY TO COMBAT PREY RESISTANCE

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Arthropod pests adversely affect humans by destroying a significant amount of the world's food supply and by transmitting numerous deadly diseases [1]. Generally, these pests have been controlled by spraying non-specific chemical insecticides. However, this is becoming increasingly ineffective due to the evolution of insecticide resistance in most medically and agriculturally important arthropods. In addition, there is a growing concern about the human health risks associated with certain agrochemicals. Thus, there is an urgent need to develop new insect control methods.

We are interested in developing highly-specific bioinsecticides by engineering recombinant insect viruses that express insect-specific peptide toxins [1]. Since the primary role of spider venoms is to kill or immobilize arthropod prey, we reasoned that spider venoms should be a rich source of insecticidal toxins. By screening the venom of the deadly Australian funnel-web spider, we discovered several families of insect-specific peptide neurotoxins that appear to be suitable for bioinsecticide development [1].

Our analysis of cDNA libraries from the venom glands of these spiders suggests that these neurotoxins are generated via a remarkable combinatorial peptide library strategy. The mature toxins are derived from a mRNA translation product consisting of an N-terminal signal sequence, a central propeptide, and a C-terminal mature toxin sequence [2]. However, rather than making the toxins as 'one-offs', the spider appears to generate a library (or 'family') of peptide toxins which sometimes vary by as little as one amino acid residue. Intriguingly, within each toxin family, there is a marked difference in the level of sequence conservation within the signal peptide and mature toxin sequences. The signal peptide, which is critical for targeting the toxin to a specific secretory pathway, is highly conserved within each family. In contrast, the mature toxin sequence is poorly conserved. It appears to have been hypermutated during the course of venom evolution, with only the cystine framework remaining conserved. By grouping functionally disparate toxins into large 'superfamilies' (which we define as toxins that contain the same signal sequence and cystine framework) we have gained significant insight into the ongoing evolutionary process by which these spiders combat prey resistance and generate peptides with novel functions.

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Biology & Evolution of Toxins - Meeting Room 1&2 - 1500-1515

ABSTRACT NUMBER 21201 1CM5

SPIDERS OPTIMISE THE USE OF THEIR VENOM

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The spider *Cupiennius salei* needs 0.01 to 10 µl venom to kill a prey item depending on kind and size of the prey. Since its venom glands contain only 10 µl and regeneration requires 8 to 16 days *C. salei* should use its venom very economically. To be able to do so *C. salei* needs two bits of information: How much venom is available in the venom glands? and how much venom does the prey need?

By a monoclonal antibody we measured, for the first time, the amounts of venom injected by a spider into different prey types. Crickets and stick insects, as victims without special defence mechanism, received only the minimum amount of venom which is not significantly different from the LD_{50}. Blowflies and ground beetles received considerably more venom because they are difficult to overwhelm or even endanger the spider by their defence behaviour. These results show that the spider is very selective with respect to the venom demand of the prey.

Next, we performed a prey-choice experiment with several cockroach species. These easily accepted prey species are especially suitable for such an experiment because the LD_{50} varies over a wide range. *C. salei* spiders had either full venom glands (control) or their venom glands were experimentally emptied by electrical milking or by allowing them to bite into a series of crickets. Two pairs of cockroaches were used: *Blatta orientalis* (LD_{50}: 0.4 nl/mg) vs. *Nauphoeta cinerea* (LD_{50}: 17.5 nl/mg) and *Periplaneta australasiae* (LD50: 0.75 nl/mg) vs. *Periplaneta americana* (LD50: 10 nl/mg). These pairs provide a difference of 40-fold and 10-fold, respectively, in venom sensitivity. *C. salei* preferred *B. orientalis* significantly over *N. cinerea* when the venom glands were empty, but not when the full amount of venom was available. No effect was evident for the other pair, indicating that a factor of approximately 10 is too low.

The combination of both results show that the spiders know how much venom is available in their venom glands and that they select prey items that are appropriate for the amount of venom available. When injecting venom, they inject very precisely the amount of venom which is needed. These results support our venom optimisation hypothesis which supposes that spiders use their venom as economically as possible.

Biology & Evolution of Toxins - Meeting Room 1&2 - 1515-1530

ABSTRACT NUMBER 12601 1CM6

THE IN VITRO NEUROTOXICITY OF VENOMS FROM 'HARMLESS' PET SNAKES

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Many colubrid snake species are popular with snake collectors and handlers. However, low venom yield and the belief that most of these snakes are only mildly venomous, or non-venomous has meant that colubrid venoms have been relatively unstudied. Therefore, the potential health implications of human envenomation by many of these snakes are unknown. Some colubrid venoms which have been examined demonstrate high protease activity, high PLA_2 activity and/or postsynaptic neurotoxic activity (e.g. Broaders et al., 1999; Hill & Mackessy, 2000). In the present study we investigated, in a skeletal nerve-muscle preparation, the in vitro neurotoxicity of venoms collected from colubrid snakes commonly sold as pets. Venoms (3mg/ml and 10mg/ml) from *Boiga cynodon, Telescopus dhara, Psamnophis mossambica* and *Coelognathus radiatus* dose-dependently inhibited indirect twitches (0.1Hz, 0.2ms, supramaximal V) of the chick biventer cervicis nerve muscle preparation. In addition, venoms inhibited responses to exogenous acetylcholine (1mM) and carbachol (20mM), but not potassium chloride (40mM). The t90 (i.e. the time taken to inhibit twitches by 90%) values of the venoms (10mg/ml; *B. cynodon*, 25 ± 8 min; *T. dhara*, 13 ± 2 min) were similar to those exhibited by highly potent elapid venoms (e.g. *Oxyuranus microlepidotus* 29 ± 3 min; Hodgson & Wickramaratna, 2003). Administration of the anticholinesterase neostigmine (5mM) at the t50 time point partially reversed, over a period of 90 min, the inhibition of twitches produced by B. cynodon venom (3mg/ml) while having less effect on other venoms. Using HPLC, we isolated an 8498 Da toxin from *Coelognathus radiatus* venom. This toxin (0-1-1mM) displayed reversible postsynaptic activity and competitively antagonised cumulative concentration-response curves to carbachol with a pA2 of 7.24 (slope 1.00 ± 0.21) determined by Schild analysis. We conclude that these colubrid venoms have postsynaptic neurotoxic activity and would like to highlight the need for caution when handling such snakes. Broaders M, Fara C & Ryan M (1999) J. Nat. Toxins., 8, 155-166. Hill RE & Mackessy, SP (2000) Toxicon 38, 1663-1687. Hodgson WC & Wickramaratna JC (2003) Clin Exp Pharmacol Physiol., 29, 807-814.

Clinical Toxinology - Meeting Room 3 - 1400-1415

ABSTRACT NUMBER 19701 1CR1

ENVENOMATION BY CAPTIVE SPECIMENS OF THE GABOON VIPER (BITIS GABONICA) – A LESSON TO BE LEARNED

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The Gaboon viper has an awesome reputation, stemming from its striking colouration, large size, enormous fangs and the remarkable quantities of venom ejected whilst biting. Although early observers claimed that the animal's bite was "perhaps more rapidly and surely fatal than that of any other snake", the literature contains not a single account of a death following envenomation with the 21 cases recorded between 1929 to 1991 all recovering fully. The principal symptomology is first, a rapid onset swelling on the bite area, which becomes very painful, then haemorrhagic oedema, dyspnoea, haematuria, haematemesis and local tissue necrosis, depending on the degree of envenomation. Despite this bleak picture, recovery after antivenom is usually complete and uncomplicated. In the last decade, the Gaboon viper has become much sought after as a captive reptile, particularly in the USA. There are a number of reasons for this popularity: the species is readily available as captive born offspring making acquisition relatively inexpensive, the snake's generally mild disposition has led suppliers to market the snake as suitable for "beginner" herpetologists and the large adult size, long fangs (the greatest length of all venomous snakes) and handsome appearance and colouration make the species an impressive reptile to display. As a result of this heightened interest, it is not surprising that an increased number of bites have occurred in recent times, including eight in the last five years of which six were in the USA. Of these, two victims were professional herpetologists, two were unlicensed collectors and four were licensed amateur herpetologists. Seven of the eight victims required hospitalisation and eventually made a complete recovery but one victim died without being able to summon help. This is the first recorded case of death as a result of *Bitis gabonica* envenomation. Post mortem examination revealed massive internal haemorrhage, congestion of the lungs and ecchymoses of the head, trunk and extremities. The seven victims who recovered displayed a range of signs and symptoms including local swelling, lymphangitis, compartment syndrome, haematuria, petechial haemorrhaging and severe coagulopathy. Changes in coagulation included severe depletion of fibrinogen, prolonged prothombin/ thrombin times and depletion of platelets, all characteristic of disseminated intravascular coagulation. Patients received between 5 and 12 vials of SAIMR antivenom and all symptoms subsided after this treatment. These case histories highlight the need for extreme care to be exercised with the handling of this species. Additionally, the increasing practice of keeping Gaboon vipers in captivity emphasises the importance of emergency physicians being fully informed on appropriate treatment strategies.

Clinical Toxinology - Meeting Room 3 - 1415-1430

ABSTRACT NUMBER 05402 1CR2

LIFE-THREATENING ENVENOMING BY THE SAHARA DESERT HORNED-VIPER (CERASTES CERASTES) CAUSING HAEMOLYTIC URAEMIC SYNDROME: MICRO-ANGIOPATHIC HAEMOLYSIS, COAGULOPATHY AND ACUTE RENAL FAILURE.

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The three species of desert horned-vipers (genus *Cerastes*) are the most distinctive, familiar and abundant venomous snakes of the great deserts of North Africa and the Middle East; from Morocco in the east, west to western Iran, north to Iraq, and south to the Sudan.

In Arabia, this is one of the most frequently observed snakes despite its nocturnal habits; in Dharan, Saudi Arabia, it was responsible for most of a series of 26 cases of snake bite and on the plains of Iraq it is the commonest snake. In Egypt, *C. cerastes* was depicted in pre-dynastic times (>3000 BC) and was represented by the phonetic hieroglyph "f" or "fy" in inscriptions and papyri. There has been long debate about whether Cleopatra used this species as the instrument of her suicide. In the 5th century AD, the Greek historian Herodotus found that at Thebes in Upper Egypt, horned-vipers were sacred; their bodies were even embalmed.

These snakes have attracted the attention of desert travellers from the time of James Bruce of Kinnaird in the 18th century to Doughty, Philby and Thesiger in the 20th century.

In modern times, *Cerastes* species have become popular among exotic-snake keepers. Two such enthusiasts were bitten on their fingers by captive specimens of Sahara desert horned-vipers (*C. cerastes*) in Europe. They developed extensive local swelling and life-threatening systemic envenoming, characterised by coagulopathy, increased fibrinolysis, thrombocytopenia, microangiopathic haemolytic anaemia and acute renal failure. The clinical picture resembled haemolytic-uraemic-syndrome, explicable by the presence in *C. cerastes* venom of several thrombin- like, Factor X-activating, platelet-aggregating, haemorrhagic and nephrotoxic components. In one case, prophylactic use of subcutaneous epinephrine may have contributed to intracranial haemorrhage.

Cerastes cerastes is clearly capable of life-threatening envenoming in humans. Optimal treatment of envenoming is by early administration of specific antivenom and avoidance of ineffective and potentially-dangerous ancillary methods. The two severely envenomed patients described would not have survived without treatment of their persistent renal failure by haemodialysis.

Clinical Toxinology - Meeting Room 3 - 1430-1445

ABSTRACT NUMBER 17701 1CR3

THE UNIQUE SYNDROME OF BERG ADDER (BITIS ATROPOS) ENVENOMING

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Bitis atropos is a small ("dwarf") adder occurring in the eastern and southern mountainous regions of southern Africa. It is both cytotoxic and neurotoxic. A prospective study of 14 cases of envenoming over a 12-year period revealed unique features not previously documented.

Apart from known clinical features, such as ophthalmoplegia, anosmia and minor cytotoxic effects, 10 patients developed respiratory failure and 9 had pronounced hyponatraemia. The respiratory failure typically developed late (8 – 36 hours post-envenoming), with a mean duration of 8 days. Hyponatraemia (down to a low of 114 mmol/l) occurred within 24 – 72 hours, with a mean duration of 5 days. In all, except 1 patient, the anosmia took weeks to months to resolve, sometimes incompletely. Similarly, pupils remained dilated for weeks to months.

Clinical evidence indicates that the hyponatraemia is probably not caused by inappropriate ADH secretion, but rather due to another unknown mechanism, possibly via a natriuretic polypeptide. The late development of respiratory failure and hyponatraemia should be anticipated, so that serious complications can be prevented. No specific berg adder antivenom is currently available.

Clinical Toxinology - Meeting Room 3 - 1445-1500

ABSTRACT NUMBER 02302 1CR4

SNAKE BITE MORTALITY IN PORT MORESBY GENERAL HOSPITAL, PAPUA NEW GUINEA (1992-2001)

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Snake bite is a leading cause of global envenoming, with an estimated annual toll of several hundred thousand deaths. Moreover, although increasing numbers of vaccine manufacturers have withdrawn from antivenom production, no international strategy has been developed to address this readily treatable injury. To facilitate priority setting for such an initiative we examined the determinants of snake bite morbidity and mortality in Papua New Guinea, a country with one of the highest rates of snake bite in the world. This study consisted of a retrospective analysis of all inpatients who died from snake bite at Port Moresby General Hospital (PMGH), the nation's major teaching and referral hospital. For the period January 1992 - December 2001 at PMGH, 88 inpatients were identified as having died from snake bite, 98% were ventilated, with a mean duration of 4.1 days. Of the 61 records analysed in detail, 54% were male, 45% were children and 77% were rural in origin. Prehospital management was reported in 31% of cases; pressure bandaging [not immobilisation] was mentioned in 32% of these, whereas 68% described the use of slashes and lacerations to the bite site with 11% using an arterial tourniquet. No snakes were formally identified and the snake venom detection kit was not used. Blood products were used in 75% of fatalities (mostly FFP) with antivenom administered in 55% [1 ampoule - 73% recieved polyvalent] with only one patient receiving multiple vials of antivenom (1 each of polyvalent and black snake). Of the 19 patients who arrived at hospital within the first four hours after snake bite, only three received antivenom within that time. Death was attributable to respiratory causes in 50% of cases (respiratory arrest, system failures such as difficulties in intubation, ventilation, bed and ventilator access or oxygen supply and overwhelming pneumonia). Probable intracerebral haemorrhage occurred in 17% of cases and renal failure in 10%. Multiple co-morbidities were present in 18% and two cases had overwhelming sepsis. In summary, deficiencies were noted at all levels of treatment of envenomed patients. Additionally, in comparison with previous analyses of mortality due to snake bite in PNG, this is a marked reduction in the ratio of patients receiving antivenom. Optimal management of snake bite therefore requires more than the provision of readily available, affordable and locally relevant antivenoms. In particular, public and professional education as well as health infrastructure development must be incorporated to address non-pharmaceutical barriers to snake bite control.

Clinical Toxinology - Meeting Room 3 - 1500-1515

ABSTRACT NUMBER 17304 1CR5

CLINICAL EVALUATION OF SNAKEBITE IN VIETNAM: STUDY FROM CHO RAY HOSPITAL

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Snakebite, a medically important problem in Vietnam, the extent of which has not been evaluated fully yet. The present study aimed to study the clinical aspects on the management of snakebites in Vietnam. From our prospective study at Cho Ray hospital, Ho Chi Minh City, 93% were bitten by venomous snakes among 129 admitted for snakebite, including *Calloselasma rhodostoma* (CR, 31%), *Trimeresurus* (T, 28.7%); *Naja naja* (NN, 25.6%) *Bungarus candidus* (BC, 4.7%) and *Ophiophagus hannah* (OH, 3.1%). The species of snakes were identified by using venom detection kit for four common venomous snakes (Le, 2003) in 33 cases. The venom levels in severe and moderate envenoming were significantly different ($P<0.05$). Venoms detected in blood of severe envenomation by CR, T, NN were 25.5 ± 16.4, 15.1± 9.2, and 35.0 ± 27.5 (ng/ml) and could be detected up to 129, 192 and 144 hours after the bites respectively. Four deaths caused by CR were due to brain haemorrhage. In contrast, neurotoxic envenomation by BC and secondary pneumonia in the period of ventilation were the reasons of two deaths. Extensive necrosis and acute renal failure were predominantly seen in *N. siamensis* bites. Antivenom for this bite was unavailable for treatment. Operations to remove necrotic tissues, amputate leg, hand or fingers and skin grafts were carried out in 23/129 cases. The mortality rate was 4.65%. In conclusion, the six common venomous snakes mainly caused the incidence in South Vietnam. Lack of antivenoms for treatment led to serious consequences with high mortality and sequalae for the surviving victims. Snake venom detection kit which was developed is useful in diagnosis and prognosis which will definitely help to improve the situation of snakebite in Vietnam.

Reference:

Le, V. D., Le, K. Q., Khoo, H. E., and Gopalakrishnakone, P. (2003). Immunogennicity of venoms from four common snakes in the south of Vietnam and development of ELISA kit for venom detection. Journal of Immunological Methods, in press.

Clinical Toxinology - Meeting Room 3 - 1515-1530

ABSTRACT NUMBER 00501 1CR6

BIOLOGICAL AND CLINICAL EVALUATION OF ANTIVENOM IMMUNOTHERAPY

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Antivenom immunotherapy is the unique specific treatment of snake and scorpion envenomations. Although widely used and medically accepted, it is still empirically administered and many questions remain unsolved. In particular, accurate guidelines are required to improve the efficacy and the safety of this treatment. ELISA tests were set up to quantify the venom toxins in patients' blood and to follow it during envenomations. Epidemiological surveys were carried out to establish clinical grading scales of viper and scorpion envenomations. Venom pharmacokinetics were followed in experimentally envenomed rabbits, in the absence of, and after antivenom immunotherapy. In a first step, ELISAs were used in parallel with clinical grading scales of viper and scorpion envenomations in France and in Tunisia. In both cases, a good correlation was observed between the venom levels in the blood and the clinical symptoms. In a second step, we examined the kinetics of the venom, in the absence of, and after antivenom administration. Such investigations were carried out in the case of envenomations by viper (*Vipera aspis* and by scorpion (*Androctonus australis garzonii*). After intramuscular injection of viper venom, the resorption of the venom follows a complex process: it is fast during the first 24 hr then it occurs at a slower rate over the subsequent 72 hr, resulting in a long half-life of elimination (36 hr). On the other hand, the absorption of scorpion toxins is very fast and complete and its half-life of elimination is short (2 hr). The effect of immunotherapy was further tested, following the kinetics of viper or scorpion venom, before and after the injection of antivenom. It appeared that the detoxification process could be explained by a redistribution of the venom from the extravascular compartment to the vascular one, where it is sequestered by the antibodies. Intravenous injection was shown to be the most effective route for antivenom administration. The relative efficacy of Fab'_2 and Fab was tested also and, in case of viper envenomations, it was shown that Fab'_2 are more efficient than Fab because of more appropriate pharmacokinetic parameters. These experimental studies provide an experimental model to optimize the antivenom immunotherapy. However, questions remain to be solved to improve further this treatment. They concern in particular the pharmacodynamic of venom toxins, their local versus systemic actions, the route of toxin elimination.

Structure/Function of Toxins - Hall C - 1600-1615

ABSTRACT NUMBER 06902 1DH1

MOLECULAR CHARACTERIZATION OF TOXINS COMPOSING THALASSOPHRYNE NATTERERI FISH VENOM

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Thalassophryne nattereri is a venomous fish responsible for severe human accidents in North and Northeast of Brazil, causing in human victims intense pain and edema followed by necrosis. The effects of *T. nattereri* venom in inflammation and blood coagulation include peculiar mechanisms, completely distinct from other animal venoms. These observations suggest that new categories of highly potent toxins might be present in this venom. Therefore, a transcriptome approach has been carried out in our labs, in order to characterize venom protein composition. A cDNA library was directionally constructed into pGEM11Zf vector from mRNA extracted from *T. nattereri* venom glands. Several clones were obtained and so far 400 were partially sequenced and searched for sequence similarity in data banks (NCBI-blastn e blastx). The analyses showed that 9% of the clones code for protein with structural function, 50% for proteins related to metabolism/protein expression/cell division and 41% display no sequence similarity. However, in this last group, 60 clones showed sequences coding for fragments that aligned with the N-terminal and internal peptides of previously isolated toxins, named natterins. These sequences were grouped in 4 distinct clusters and the clones having the longest inserts from each group were amplified, completely sequenced and cloned into expression vectors. Recombinant natterins 1 and 2 have already been obtained in *E. coli*, using T7 based promoter vector, as inclusion bodies. By western blotting, both proteins strongly reacted with *T. nattereri* antivenom produced in horses, confirming their nature as venom components. Refolding processes have been attempted in order to obtain the toxins with conserved biological activity. Recombinant natterin 2 have induced a rapid thrombus formation and immediate stasis in venules, as observed for crude venom. The expression of other selected cDNAs are underway and might reveal biological activity unrelated to natterin 2. Moreover, the generation of more ESTs may help to elucidate venom composition, an important step to understand the clinical aspects of the local lesion aiming the development of a rational treatment of its victims.

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Structure/Function of Toxins - Hall C - 1615-1630

ABSTRACT NUMBER 10101 1DH2

IMPORTANCE OF THE CONSERVED AROMATIC RESIDUES IN THE SCORPION ALPHA-LIKE TOXIN BMK M1: THE HYDROPHOBIC SURFACE REGION REVISITED

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About one third of the amino acid residues conserved in all scorpion long-chain Na^+ channel toxins are aromatic residues, some of which constitute the so-called "conserved hydrophobic surface". At present, in depth structure-function studies of these aromatic residues using site-directed mutagenesis are still rare. In this study an effective yeast expression system was used to study the role of seven conserved aromatic residues (Y5, Y14, Y21, Y35, W38, Y42 and W47) from the scorpion toxin BmK M1. Using site-directed mutagenesis, all of these aromatic residues were individually substituted by glycine in association with a more conservative substitution of phenylalanine for Y5, Y14, Y35 and W47. The mutants, expressed in *S. cerevisae* S-78 cells, were then subjected to a bioassay on mice, an electrophysiological characterization on cloned Na^+ channels (Nav1.5) and a CD analysis.

Our results show an eye-catching correlation between the LD50 values on mice and the EC50 values on Nav1.5 in oocytes, indicating large mutant-dependent differences that emphasize important specific roles for the conserved aromatic residues in BmK M1. The aromatic side-chains of the Y5, Y35 and W47 cluster protruding from the three-stranded beta-sheet, seem to be essential for the structure and function of the toxin. Residues W38 and Y42, respectively located in beta2 and in the loop between beta2 and beta3, are most likely involved in the pharmacological function of the toxin.

Structure/Function of Toxins - Hall C - 1630-1645

ABSTRACT NUMBER 23301 1DH3

EXPRESSION OF OMWAPRIN IN E.COLI, A MEMBER OF WAPRIN FAMILY OF SNAKE VENOM PROTEINS

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Omwaprin is a small protein consisting of 50 amino acids with a mass of 5602.52 Da. purified from the venom of Inland Taipan (*Oxyuranus microlepidotus*). It belongs to the Waprin family (Whey acidic proteins related proteins) a new family of snake venom proteins recently identified by our group. It has eight conserved cysteine residues similar to other four-disulphide core proteins like SPAI, EXPI, Elafin, KAL-1 etc. Since Omwaprin is found in small quantities in venom we planned to express it in *E.coli* using a synthetic gene. We have designed and constructed a synthetic gene using *E.coli* preferred codons.We were able to express the protein in *E.coli* in a soluble form in reasonably good amount by using pET32a as expression vector. The molecular weight of the recombinant Omwaprin using ESI Mass Spec. matched with the calculated mass of 5602.5. Recombinant Omwaprin shows similar folding as indicated by its elution on a reverse phase HPLC column.

Structure/Function of Toxins - Hall C - 1645-1700

ABSTRACT NUMBER 19502 1DH4

HETERODIMERIC DISINTEGRINS IN VIPERA LEBETINA SNAKE VENOM GLAND ARE SYNTHESIZED FROM DIFFERENT GENES

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Crotalid and Viperid venoms contain a large number of metalloproteinases. Most venom metalloproteinases are multidomain enzymes built up by an N-terminal metalloproteinase domain, C-terminal disintegrin or disintegrin-like domain and cystein-rich domain. The cDNA of the snake venom disintegrin family precursor usually encodes prepro-peptide, metalloproteinase, spacer, and disintegrin domains. It is supposed that disintegrins are synthesized in venom gland as a domain of a metalloproteinase and are released by proteolytic processing. Different disintegrins have been purified from *Vipera lebetina* and *Vipera lebetina obtusa* venom (Gasmi et al. 2001, Eble et al. 2003, Calvete et al. 2003). We have shown that the disintegrin-like domain observed from the cDNA sequence of lebetase from Central Asian *V. lebetina* venom is processed away and lebetase is purified without disintegrin-like part from the venom (Siigur et al. 1996). Heterodimeric disintegrin VLO5 from *Vipera lebetina obtusa* venom (Calvete et al.2003) contains the chain that has identical sequence with lebetase disintegrin-like domain with VGD motif. We have also isolated from the *V. lebetina* venom gland cDNA library a cDNA encoding only the disintegrin domain and lacking the metalloproteinase domain. The translated protein sequence of this RGD-containing disintegrin is exactly the same as previously sequenced for lebein alpha chain (Gasmi et al. 2001). The alpha subunit of the heterodimeric disintegrin lebein has a short coding region. Recently it was demonstrated that such short coding region occurs also in heterodimeric disintegrin acostatin from the venom of *Agkistrodon contortrix contortrix* (Okuda et al.2002). Most probably dimeric disintegrins are synthesized from different genes. We detected the structural identity of isolated disintegrins with deduced protein sequence on the basis of MALDI-TOF MS analysis of tryptic fragments of disintegrins. We have cloned *V.lebetina* venom metalloproteinase factor X activator (VLFXA). VLFXA heavy chain contains the disintegrin like domain with DECD motif. The disintegrin-like domain is not processed away in this case. We have also isolated some other clones of ADAM –type metalloproteinases with disintegrin-like domains from cDNA library of *V. lebetina* venom gland.

Ref. : Calvete et al. Biochem. J. in press BJ20021739 (2003); Eble et al. J. Biol. Chem. in press M301860200 (2003); Gasmi et al. Biochim. Biophys.Acta 1547, 51 (2001); Okuda et al. Biochemistry 41, 14248 (2002); Siigur et.al. Biochem. Biphys. Res. Comm.224,229 (1996); The work was financially supported by Estonian Science Foundation grant No. 5554.

Structure/Function of Toxins - Hall C - 1700-1715

ABSTRACT NUMBER 07601 1DH5

PHOTODYNAMICS OF SNAKE VENOM PROTEINS USING UV SENSITIZED METHYLENE BLUE

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Photodynamics are the changes caused by photochemical reactions between light absorbing molecule and the protein, drug or chemical entity. The reaction which induces alterations due to absorption of radiation energy include, oxidation-reduction, isomerisation, ring rearrangement, modification, polymerisation of chemical or drug molecule and alterations in the toxophoric group or selective perturbations in the protein molecule. Various methods for photooxidation of venom protein used to generate photooxidised product of immunological significance or for photodynamic studies which include, γCo^{60} radiation, direct exposure to UV light, visible and UV light in the presence of methylene blue etc. Methylene blue revealed biphasic spectrum with absorption maximum at wave length region 292 nm and 615 nm. Increase in the absorbance was used as an indicator for the generation of photooxidised venom product (POVP) of *Naja saimensis* (Gawade, 2000) and *Echis carinatus* (Prasher, 2002). Absorption characteristics of POVP of *Echis carinatus* venom protein revealed statistical significance (p = 0.018,F=2.386). UV spectral analysis of POVP showed, flattened region at 240 to 280.6 nm due to transitions in amide backbone. Loss of peak in 1st and 2nd Deriv. DT=1, was suggestive of the transitions in amide backbone and aromatic amino acids respectively. Survival time of mice was prolonged to more than 2 days, as compared to mortality in 30 and 156 min.following intracerebral and intramuscular injections. Venom proteins showed prominent analgesic, anticonvulsant, depressant, mild muscle relaxant property besides,marked phospholipase A2 and fibrinogen clotting activity. Whereas POVP exhibited algesic and antidepressant property as evidenced from the increased number of acetic acid induced writhings [10.67 ± 1.4 to 13.67 ± 2.9, n = 6],prolongation of sleep onset [6.5 ± 1.15 to 9.2 ±1.35 min.n=6] and shortened duration of pentobarbitone induced sleeping time [16.8 ± 0.6 to 15.3 ± 1.52 min. n=6]in mice. POVP however, showed very weak precipitin reactivity on agar gel diffusion. The generation of biologically active photooxidised species and the significance of alterations in the pharmaco-photodynamic research will be presented.

Gawade,S.P.(2000): Photodynamic action of UV sensitized methylene blue on the venom of Thialand cobra *Naja saimensis*. J.Venomous Animals & Toxins, 2,6:271-278.

Prashar S. (2002): Pharmacological studies on photooxidised *Echis carinatus* venom using methylene blue and screening of herbal drug for antivenom property in rats. M.Pharm.(dissertation) Pharmacology, RGUHS, Bangalore. Karnataka,India.

Structure/Function of Toxins - Hall C - 1715-1730

ABSTRACT NUMBER 15401 1DH6

CHARACTERIZATION OF AMM VIII FROM ANDROCTONUS MAURETANICUS MAURETANICUS: A SCORPION TOXIN WHICH DISCRIMINATES BETWEEN NEURONAL AND SKELETAL SODIUM CHANNELS

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The venom of the scorpion *Androctonus mauretanicus* was screened using ELISA with a specific serum directed against the alpha-toxin of reference, AaHII. This led to the isolation of AmmVIII (7382.57 Da). It gave a highly positive response in ELISA, but was totally devoid of toxicity when injected subcutaneously in mice. In voltage-clamp experiments, on the contrary to AaHII which affects with nearly the same potency the rat brain type II voltage-activated Na^+ channel (rNav1.2) or rat skeletal muscle voltage-activated Na^+ channel (rNav1.4) expressed in *Xenopus* oocytes (EC50 value = 2.6 nM and 2.2 nM respectively), AmmVIII differently modifies rNav1.2 and rNav1.4 with a significant lower affinity and efficacy (EC50 value = 29 nM and 416 nM respectively). The AmmVIII cDNA was PCR amplified and its oligonucleotide sequence determined. AmmVIII shows 87 % amino acid sequence homology with AaHII, but carries an unusual extension at its C-terminal end, consisting of an additional Asp due to a point mutation in the penultimate codon. This extra amino acid residue could induce steric hindrance and dramatically reduce recognition of the target by AmmVIII. Comparison of 1H Nuclear Magnetic Resonance spectra of AmmVIII and AaHII, together with the recording of 1H 2D-NOESY spectrum for AmmVIII reveal that AmmVIII folds in the same way as does AaHII. This demonstrates that the C-terminal extension does not lead to an overall conformational change in the AmmVIII 3D structure. Using the AaHII 3D X-ray structure as template, we constructed accordingly a model of AmmVIII to clarify the reduction in activity. However, the few sequence differences also found in the AmmVIII amino acid sequence drastically modify the charge repartition and the electrostatic dipole moment of the molecule. Liquid phase radioimmunassays with polyclonal and monoclonal AaHII antibodies indicated the loss of conformational epitopes between AaHII and AmmVIII.

Biology & Evolution of Toxins - Meeting Room 1&2 - 1600-1615

ABSTRACT NUMBER 11601 1DM1

DETECTION OF TOXINS BY ELECTROCHEMICAL LUMINESCENCE.

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Electrochemical luminescence (EL) is evolving into a superior detection technology. It offers an unparalleled combination of speed, sensitivity, and flexibility to tailor specific solutions to different assay problems. We used the M8 ECL analyzer (Igen International) to develop sensitive assays for a variety of plant and bacterial toxins. Signal generation is based upon a stable ruthenium chelate. An electrode-activated catalytic process triggers a light reaction such that the ruthenium chelate emits a 620nm wavelength. Because few compounds fluoresce at this wavelength, background is low and signal:noise ratios are excellent. The M8 analyzer uses a flow-through format based upon strepavidin-coated paramagnetic beads. This offers the advantages of solution-phase kinetics, elimination of wash steps, and faster assay times. We have developed antibody-based ECL assays fro several serotypes of botulinum toxins, staphlococcal enterotoxins, and ricin. These assays provide excellent sensitivity in a fraction of the assay time of colorimetric capture ELISAs. In addition, we adapted the ECL technology to monitor the enzymatic activity of botulinum neurotoxins and the lethal factor of Bacillus anthracis. These functional assays have practical applications in the quest for specific toxin inhibitors as potential therapeutic agents.

Disclaimer: opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the US Army.

Biology & Evolution of Toxins - Meeting Room 1&2 - 1615-1630

ABSTRACT NUMBER 08902 1DM2

OSTREOLYSIN, A CYTOLYTIC PROTEIN FROM THE EDIBLE MUSHROOM PLEUROTUS OSTREATUS AND ITS INTERACTION WITH LIPID MEMBRANES

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Ostreolysin is an acidic 15 kDa cytolytic protein specifically expressed during frutification of the edible mushroom *Pleurotus ostreatus*. Recently, entire sequence of ostreolysin deduced from the c-DNA library has been elucidated. Although there are some minor differences compared to the sequence of first 50 aminoacid residues obtained by protein sequencing, the c-DNA derived sequence shows 80% identitiy and also a large degree of homology with the protein sequence derived from Aa-Pri1 gene expressed during primordia and fruiting initiation of the mushroom *Agrocybe aegerita* and Asp-hemolysin from *Aspergillus fumigatus*. Ostreolysin is strongly hemolytic and readily disrupts vesicles reconstituted from the erythrocyte membrane lipids. At nanomolar concentrations, the protein lyses human, bovine, and sheep erythrocytes apparently by a colloid-osmotic mechanism, compatible with the formation of pores of 4 nm in diameter. Ostreolysin was also cytotoxic to mammalian tumor cells. Cytolytic activity of ostreolysin is strongly inhibited by lysophospholipids and fatty acids at the concentrations well bellow their CMC. However, inhibition of ostreolysin by lysophospholipids is not correlated to their role in binding ostreolysin to the membranes. Interaction of ostreolysin with lipid vesicles and their permeabilization correlated with an increase in a-helical structure of the cytolysin but was independent of the lysophospholipid content of the vesicles. It appears that either an unknown lipid acceptor or a specific lipid complex (i.e lipid raft) is required for binding, aggregation and pore formation.

Biology & Evolution of Toxins - Meeting Room 1&2 - 1630-1645

ABSTRACT NUMBER 09402 1DM3

DIVERSE TOXICOLOGICAL MANIFESTATIONS DUE TO DIFFERENT SPECTRA OF FUNGAL TOXINS IN 2 MYCOTOXICOSES CAUSED BY ASPERGILLUS SPP. IN ISRAEL

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It is well known that many fungal spp. produce toxins to repel, harm or kill biological organisms with the potential to harm them. Several important principles of mycotoxins that are less well appreciated that may make recognition of mycotoxicosis more difficult. Firstly, the fungi invariably produce more than one toxin. For example, in a limited study in Germany, samples of wheat grains were examined for just 10 tricothecenes mycotoxins out of the > 100 known to be produced by *Fusarium* spp.. Over 70% contained 3-5 toxins and 5% contained more than 6 of the 10. These toxins vary in their toxicity and even target organ. The amount of the different toxins produced may also vary, probably according to the fungal strain and environmental conditions. Also there may be interactions between the toxic effects of the toxins, often as a synergism. These facts may bring about a state whereby a certain spectrum (number, types, concentrations, interactions) of toxins may culminate in different clinical manifestations than another spectrum produced by the same fungal sp. This theory is demonstrated by 2 mycotoxicoses in Israel, a chronic *Aspergillus clavatus* toxicosis in sheep, and a sub-acute probable *Aspergillus* sp. toxicosis in poultry.

The occurrence in sheep in Israel was manifested by a lethal 'neurotoxic syndrome consequent to feeding barley sprouts contaminated with *A. clavatus*. Toxicoses caused by *A. clavatus* toxins in sheep in England and South Africa had quite different characterizations, with the toxicosis in South Africa being somewhat similar to the other 2 cases, but the manifestations of the Israeli and English cases being quite dissimilar. A movie to be shown depicts this unique toxicosis. Similar cases have been recently reported in Australia after feeding grains from beer production to cattle.

The mycotoxicosis in poultry was induced by feeding corn in which ochratoxin was found at concentrations of 300-700 μg/kg. Despite the fact that ochratoxin is a characteristic nephrotoxin, affected geese showed severe hepatotoxicity with only mild kidney damage, whereas broilers showed severe ascites, hepatotoxicity and no kidney lesions. These findings may be explained by the report of feeding 4 isolates of *A. ochraceus* to chicks. Two of the strains produced ochratoxin and caused mortality, whereas 2 other strains did not produce ochratoxin, and yet still caused mortality, characterized by hepatopathy and ascites. This indicates that in the Israeli case, ochratoxin may have had a lesser role than an unknown hepatotoxin produced by some *A. ochraceus* strains, and served as an indicator for unknown toxins.

Biology & Evolution of Toxins - Meeting Room 1&2 - 1645-1700

ABSTRACT NUMBER 20802 1DM4

EFFECTS OF MICROCYSTINS ON RAT AND HUMAN HEPATOCYTES

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Microcystins are potent hepatotoxins and tumor promoters. Exposure of hepatic cells to microcystins leads to compaction of cytoskeletal proteins, and to apoptosis. It is well known that acute lethal doses induce intrahepatic hemorrhage, but chronic administration of sub lethal doses is less studied, although such intoxication is more probable in humans. The aim of the study was to assess the effects of microcystin LR (MCLR) using laser scanning confocal microscopy for evaluation of the effects on a molecular level, and MRI for the effects in vivo. Male Wistar rats weighing 250 to 300 g were used in all MRI experiments. Rats were treated with MCLR i.p. every second day for three weeks. During the treatment they received 2 LD50 of MCLR. The other group of experimental animals received 2 LD50 i.p. as a single dose. MRI of liver was performed on experimental animals under anesthesia on 2.35 T Bruker Biospec system, operating at 100 MHz. Multi slice multi echo technique was used to obtain T1-weighted images. Slice thickness 2 mm, RT 450 ms, TE 30 ms. Liver samples from sacrificed animals as well as cultured rat and human hepatocytes were examined by laser scanning confocal microscopy (Leica, Germany). Actin filaments were stained with rhodamin-phaloidin, and nuclei with sytox green. Acute intoxication resulted in an increase of liver volume, and subchronic intoxication caused morphological changes of liver that were detected by use of MRI, laser scanning confocal microscopy, and by histological analysis of bioptic samples. MRI of the liver form control animals showed homogeneous dark-grey color. After acute intoxication with MCLR liver volume increased for 24.1 ± 2.7 %. Subchronical treatment with MCLR resulted in a less homogeneous appearance of the liver with diffuse and widespread hyper-intense regions without liver volume changes. Pathohistologic examination revealed intrahepatic hemorrhage in the case of acute intoxication, and parenchymatose degeneration with periportal fibrosis after the sub chronic exposure of animals to MCLR. In vitro MCLR caused separation of cells, condensation of nuclei, and compaction of actin filaments. Hyper intense regions observed on the MR images from the animals treated with MCLR for the period of three weeks were characterized pathohistologically as a degenerative processes. There was also a good agreement between the MRI data and findings on cellular level detected by the laser scanning confocal microscopy. The data obtained from animals sub chronically exposed to MCLR indicate that MCLR may produce degenerative processes that may lead either to apoptosis or to formation of neoplasms, depending on the duration of exposure and the dose of microcystins used in experiments. The results allow the conclusion that sub chronic application of MCLR causes changes in rat and human hepatocytes. MRI can reliably detect these changes in liver in vivo.

Biology & Evolution of Toxins - Meeting Room 1&2 - 1700-1715

ABSTRACT NUMBER 00601 1DM5

CHARACTERIZATION AND CRYSTALLOGRAPHIC STUDIES OF EMS16, AN ANTAGONIST OF COLLAGEN RECEPTOR (GPIA/IIA) FROM THE VENOM OF ECHIS MULTISQUAMATUS

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EMS16 is a member of the snake venom derived C-type lectin family of proteins (CLPs) found in the venom of *Echis multisquamatus*. It binds to the glycoprotein Ia/IIa (integrin $\alpha 2\beta 1$), a major collagen receptor of platelets, acting as a potent antagonist of platelet aggregation and cell migration. Amino acid sequencing and cDNA cloning of EMS16 have revealed that it is composed of 134 amino acid residues in the A chain and 128 residues in the B chain. The deduced amino acid sequence indicates that the 21st residue of the B chain is Asn. Alignment of the 21st to 23rd residues (Asn-Trp-Thr) suggests the possibility of N-glycosylation of the Asn at position 21 in the B chain, which also agrees with the result of amino acid sequencing of the B chain, in which the 21st residue could not be identified. Crystals of EMS16 belong to the space group $P2_12_12_1$, with unit-cell parameters a = 46.57, b = 59.93, c = 115.74 Å, and diffract to a resolution of 1.9 Å. Phase determination is underway using molecular replacement with the structure of blood coagulation factor IX-binding protein (IX-bp) from habu snake venom (PDB code 1bj3) serving as the search model.

Biology & Evolution of Toxins - Meeting Room 1&2 - 1715-1730

ABSTRACT NUMBER 11201 1DM6

NOVEL HETEROLOGOUS C3 CONVERTASES FROM TRIMERESURUS FLAVOVIRIDIS VENOM THAT INDEPENDENTLY CLEAVE HUMAN C3 AND INITIATE THE COMPLEMENT CASCADE

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The anaphylactic shock from the habu snake (*T. flavoviridis*) bites gave irreparable damage to victims and may be caused by massive releases of C3a and C5a in complement system. We have shown a strong activation of the human alternative complement cascade through formation of C3a and C5a terminating in the formation of a membrane attack complex by some protein component(s) in the *T. flavoviridis* venom. To identify the responsible factor(s), the crude venom was fractionated by serial chromatographies and the active fractions were evaluated by C3a-releasing and soluble membrane attack complex-forming activities. Two peak fractions with the highest activities were detected after gel filtration and ion exchange chromatography, and the first fraction was purified to homogeneity. The N-terminal sequence of 25 amino acids completely coincided with that of previously reported serine protease, flavoxobin, with low coagulant activity. The digesting pattern revealed that flavoxobin cleaves a chain of C3 into two fragments. The N-terminal amino acid sequences for remnant fragments of C3 disclosed that flavoxobin cleaved human C3 at the Arg726-Ser727 site to form C3b and C3a similarly as human alternative C3 convertase did. In conclusion, flavoxobin acts as a novel, heterologous C3 convertase that independently cleaves human C3 and kick-starts the complement cascade.

Clinical Toxinology - Meeting Room 3 - 1600-1615

ABSTRACT NUMBER 09903 1DR1

SUB-CLINICAL ALTERATIONS IN VICTIMS OF SCORPIONISM WITH ONLY LOCAL SYMPTOMS AND THE NEED TO DETECT VENOM QUICKLY.

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We have analysed plasma from 164 victims of *Tityus discrepans* sting who developed only local symptoms, looking for early markers of envenomisation. We studied the plasmatic levels of enzymes, cytokines, tumour necrosis factor, and nitric oxide. We found that 70 % of patients with only local symptoms showed important venom concentrations. We showed that 15 % of the cases were procoagulated showing low partial thromboplastin time (PTT) and 13 % were anticoagulated presenting PTT values above normal. Hyperamilasemia was observed in 34 % of the patients and 12 % of them were hyperglycaemic. We found increased plasmatic concentrations of NO, IL6, TNF-a and IL1-a in 53%, 42%, 32% and 16% of cases respectively. Our results demonstrated that *Tityus* venom is able to induce inflammatory and coagulatory disorders which remain sub-clinical until organ's reserve capacity is exhausted. Based on our findings and trying to provide a rational basis for a quickly therapeutic management, we developed a lateral flow immunochromatographic test able to detect in 15 min the presence of venom in blood. This test will be relevant in cases where symptoms are not developed due to low venom concentrations, or in places where doctors are not familiarised with the clinic symptoms of scorpionism, or with patients that are no able to refer the origin of envenomisation. This work was partially supported by grant IVIC/SILANES N° 2001-31.

Clinical Toxinology - Meeting Room 3 - 1615-1630

ABSTRACT NUMBER 05902 1DR2

SCORPION ENVENOMING AND TREATMENT IN MEXICO

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The climatic and geographical situations of Mexico provide excellent conditions for scorpion speciation. Mexico has in excess of 200 different species of scorpions. Some species are very abundant and have domestic habits, explaining the high level of human accidents due to scorpion stings. The Pacific Coast and a Central area of Mexico are the regions where the most dangerous scorpions thrive (8 species and subspecies of the genus Centruroides, distributed in 13 endemic States of the country). Since the medical notification became obligatory, the number of cases published in the Epidemiological Surveillance Bulletin of the Ministry of Health increased from 83,814 in 1995 to 170,115 (1997), 208,444 (2000) and 229,713 (2002), confirming earlier suspicions (Dehesa-Davila and Possani, Toxicon, 32:1018, 1994). Concurrently, the number of deadly cases decreased from 230, 140, 84 and 70, respectively for years 1995, 1997, 2000 and 2002, which represents a decrease in the mortality rate from 0.27, 0.08, 0.04 and finally to 0.03 during the same corresponding years. This apparent paradox (increasing number of stings and decrease of mortality) is due to improvement of registration efficiency by hospitals and medical centers, and to the increment in the use of antivenom. A general campaign was lunched in the country to encourage the medical community to use the excellent horse anti-venom available, which is distributed in a lyophilized form, after purification and enzymatic cleavage of the immunoglobulins [F(ab')2 fragments]. This biological product has been consistently shown to be an excellent and safe medicament to be used in all cases of patient presenting with one or more of the systemic symptoms listed here: irritability, hyperthermia, local pain, paresthesia in the entire body, nasal pruritus, pharyngeal sensation of foreign bodies, syalorhrea, sneezing, tongue fasciculation, labial cyanosis, sweating, photophobia, nistagmus, alterations of cardiac and arterial pressure, abdominal distention, respiratory problems, vomiting, diarrhea, lung edema, convulsions and priapism. Cases with local symptoms only (local pain and pruritus, sometimes accompanied by local paresthesia and light edema on the site of sting or hyperemia) do not require application of the antivenom. Time elapsing from sting and treatment are crucial for rapid recovery of the patients. Usually one vial doses applied in duly time is enough to circumvent the intoxication phenomenon.

Acknowledgements: Supported in part by grants received from CONACyT (Z-002 and Z-005) and Instituto Bioclon S.A. de C.V.

Clinical Toxinology - Meeting Room 3 - 1630-1645

ABSTRACT NUMBER 10901 1DR3

CARDIOPULMONARY EFFECTS OF THE VENOM OF PARABUTHUS SCORPION SPECIES IN THE ANAESTHETISED PIG MODEL

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This study was designed to determine the contribution of cardiopulmonary pathology to the overall clinical picture of *Parabuthus* scorpionism.

Thirteen pigs were given *P. granulatus* and eight *P. transvaalicus* venom intravenously (n=21). General haemodynamic parameters were recorded for both groups and included heart rate (HR), mean aortic blood pressure (MAP), pulmonary artery pressure (PAP), and cardiac output (CO). Derived haemodynamic parameters were calculated for both groups and included left ventricle (LV) pressure and volume, LV end-systolic pressure-volume relations (Ees), and preload recruitable stroke work, all indexes of LV contractility. Pulmonary parameters included arterial and mixed venous blood gases, pulmonary compliance, shunt (Qs/Qt), and dead space (Vd/Vt). Plasma catecholamines were measured and oral secretions recorded and graded. Measurements were taken before and at 3, 12, 30 and 60 minutes post venom injection. Lung tissue was obtained for histology before and after 60 minutes of venom injection. Data was analysed with repeated ANOVA and regression performed with the method of least squares.

The haemodynamic and pulmonary parameters showed numerical but no statistical differences. However, results indicated a trend of increased HR and MAP in both groups. In the P. transvaalicus group, the noradrenalin levels increased, while it remained constant in the P. granulatus group. There was no correlation between the change in haemodynamic parameters and the change in noradrenalin levels. In both groups, copious oral salivation was significant. Lung histology before and after venom injection indicated no pathological change.

In conclusion, in view of the unchanged myocardial contractility and unchanged pulmonary artery pressures, together with unchanged lung histology, it seems that parasympathetic stimulation, and not pulmonary oedema, is the cause of respiratory distress and failure in the *Parabuthus* envenoming syndrome.

Clinical Toxinology - Meeting Room 3 - 1645-1700

ABSTRACT NUMBER 09901 1DR4

MODELING TITYUS SCORPION VENOM PHARMACOKINETICS: IMPLICATIONS FOR THERAPEUTICS

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We measured pharmacokinetic parameters for *Tityus discrepans* in rams. The venom was injected s.c. in the inner side of the thigh in doses of 40, 75 o 100 µg/kg. The plasma venom content (venenemia) was determined with an specific sandwich type ELISA from 0 to 300 min after injecting venom. Venenemia was fit to a 3 compartment model (inoculation site, plasma and extra vascular space), it was assumed that the venom may also be irreversibly removed from plasma. The following equations were solved numerically in the *ADAPT II* Pharmacokinetic/Pharmacodynamic Analysis Software (D'Argenio and Schumitzky, 1997):

$$\frac{dM_p}{dt} = k_a \cdot M_{inoc} + k_{ev} \cdot M_{ev} - \left(k_{pe} + k_{el}\right) \cdot M_p ; \frac{dM_{inoc}}{dt} = -k_a \cdot M_{inoc} ; \frac{dM_{ev}}{dt} = k_{pe} \cdot M_p - k_{ep} \cdot M_{ev}$$

Where the amounts of venom (mg) are: M_{inoc}= inoculation site; M_p= plasma, M_{ev}= extra vascular space. The diffusion rate constants (min^{-1}) are: k_{pe} = from plasma to extra vascular space, k_{ep} = from extra vascular space to plasma. k_{el} = rate of venom elimination and k_a = diffusion from inoculation site to plasma. We calculated the plasmatic [11,17 (6,62, 16,58) lit, median and 95% CI] and extra vascular [215.8 (119.9, 1766.8) lit] distribution volumes. The values were (5 rams) : k_a = 1.29 (0.97, 1.79)·10^{-2} min^{-1} , k_{el} = 1.15 (0.60, 2.95)·10^{-3} min^{-1}, k_{pe} = 1.75 (0.52, 8.69)·10^{-2} min^{-1} y k_{ep} = 1.05 (0.28, 2.6)·10^{-3} min^{-1}, V_p =, V_{ev}=. We calculated the time course of venom content in the 3 compartments and found that at any time no more that 30% of the venom is present in plasma, peaks at 1h and decays afterward. The venom content in the injection site decays exponentially for over 6 h; this prediction was confirmed immunohistochemistry. Only ≤5% of the venom is eliminated in up to 10 h, ≥80% of the venom is in the tissues after 2 h and remains there for >10 h when the venenemia is negligible. The plasma compartment is a transient pathway from the envenoming site to the target organs, therefore venenemia underestimates the amount of venom received; this underestimation grows with envenoming time. These results and studies in course where $F(ab')_2$ therapy is introduced in the model, remark the importance of the early use of anti venom ideally during the first hour of envenoming . This work was partly financed by the Laboratorios Silanes (Mexico).

Clinical Toxinology - Meeting Room 3 - 1700-1715

ABSTRACT NUMBER 22702 1DR5

PAPUAN TAIPAN (OXYURANUS SCUTELLATUS CANNI) ENVENOMATION IN RURAL PAPUA NEW GUINEA

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The management of elapid snakebite can be problematic in even the most well resourced Intensive Care facilities. In Papua New Guinea where chronic decline in health care infrastructure, limited staff training and expertise already complicate the provision of care, risks are also increased by factors such as extremely poor transport infrastructure, communications and climate that have little to do with clinical issues, but may have tremendous impact on clinical outcomes. Nine illustrative cases of serious envenomation following bites by Papuan taipans *Oxyuranus scutellatus canni* are presented. Eight cases were fatal with contributing factors such as delayed presentation with advanced symptoms, incorrect antivenom administration, failure to recognise key indications of envenoming, lack of antivenom, and delayed administration of correct antivenom. These are contrasted by the survival of a single patient after prompt recognition of coagulopathy as a symptom of envenoming, followed by administration of appropriate antivenom and careful patient monitoring using the 20WBCT protocol. Although rural health centres operate under basic conditions without modern medical equipment or reliable power they are staffed by dedicated health workers who accept these limitations and endeavour to provide communities with the best possible medical services. A consistent approach to practical clinical management incorporating strategies such as early recognition of envenomation, prompt pressure immobilisation, precise ongoing clinical assessment and early treatment with appropriate antivenom needs to be developed and established at all rural health centres. The prognosis for envenomed patients would also be improved by public education, and appropriate ongoing health worker training.

Clinical Toxinology - Meeting Room 3 - 1715-1730

ABSTRACT NUMBER 13201 1DR6

DEATH BY BOTHROPIC ACCIDENT. PRESENTATION OF A CASE

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Background: tissue damage at the site of the bite and coagulation disturbance are the most common manifestation following envenoming by a South America *Bothrops* snake. This effect is multifactorial, including proteases enzymes, hyaluronidase and phospholipase A_2 actions, mediator of the inflammatory response liberation, pro-coagulate effects and hemorrhagic toxins (hemorrhagins) that damage basement membrane of vessel walls. The manifestations of cytolytic envenoming are local pain, redness, swelling, ecchymosis, hemorrhagic blebs, not only in the area around the bite, for in the severe cases, may involve all the affected limb, occasionally with elevated muscle compartment pressures and compartment syndrome. *Bothrops* venoms may activate coagulation factor X and prothrombin and lyse fibrinogen, causing hypofibrinogenemia or complete fibrinogen consumption which can result in partial or complete blood incoagulability. Death by Brazilian *Bothrops* bite is rare in Brazil. We present a case of a patient who died from a bothropic accident who, after a compartment syndrome, developed several complications. Case: D.C.S., a 49-year-old male farmer, who was working in Nazare Paulista, SP, was attended at the Hospital in Atibaia two hours after having been bitten three times by a *Bothrops* snake in the left deltoid region, with intense local pain, redness, and swelling, involving not only the forearm, but the whole limb as well. The snake was on a bush about 1.20 m from the ground. The patient received 15 vials of *Bothrops* antivenoms serum about three hours after the accident. The accident occurred at 0:30 p.m., on 04/19/2002, but only at 1:15 p.m. of April 20, the Poison Center was contacted, and he arrived at the University Hospital at 5:25 p.m. On arriving, he presented a severe compartment syndrome, with an acute swelling of the whole upper left limb, without radial, ulnar pulse, and perfusion confirmed by the Doppler, rigidity, cyanosis, and lack of sensitivity and mobility in the left hand and in the distal part of the forearm. After diagnosis of the compartment syndrome, the patient underwent a fasciotomy under general anesthesia. Areas of necrosis in the anterior musculature of the forearm were observed. He was intubated with a mechanical ventilation until the early hours of April 22, when he was extubated. His vital signs were maintained near normal until the early hours of April 24, when he presented with abdominal distention and intestinal hemorrhage, which was clinically treated until April 26, when he developed acute perforative abdomen and underwent an exploratory laparotomy when a perforated duodenal ulcer and intestinal hemorrhage were found. A vagotomy, with subtotal gastrectomy, duodenostomy and jejunostomy, were performed, in addition to a surgical debridement of the left limb injuries. Due to a digestive hemorrhage, clinically uncontrollable, he underwent further surgery on May 4, and the abdominal cavity was closed with a screen, only. The patient died on May 8, from multiple organ failure. Comment: this has been the first case of death by bothropic accident recorded at the Poison Center since it opened in 1982.

Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 04101 1PF.1

THE REPORT OF THE EMERGENCY TREATMENT WITH RESPIRATORY ARREST DUE TO CHINESE BANDED - KRAIT (BUNGARUS MULTCINCTUS) BITE

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We report the emergency treatment in 62 cases (48 of men, 14 of woman) of many system organ failure (M.S.O.F.) due to Chinese Banded-krait bite during 1974-2001. In those cases, there were 53 cases (42 of male, 11 of female) with success in treatment and, on the other hand, 9 of death (6 of male , 3 of female). Use trachea intubation in 42 cases, tracheotomy in 48 cases, artificial respiration in 51 cases, simple respirator of oxygen supplying with anestheasia machine in 11cases. In the early stage of krait-bite, there were no significant symptoms to be recognized. So, usually, both the patients and doctors might neglect its serious consequences. Among these 62 cases on admission, only 35 cases of them could be diagnosed correctly. Therefore, early diagnosis must be the first step to success in emergency treatment.

Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 06802 1PF.1

IN VITRO ANTIBACTERIAL STUDIES OF CRUDE EXTRACTS AND ISOLATED COMPOUNDS FROM TRAGIA INVOLUCRATA AS A TRIBAL MEDICINE IN INDIA

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The efficacy of *Tragia involucrata* is well known in Indian traditional medicinal systems. Tribes in Tamilnadu, Western Ghats of India are using different parts of this plant for skin infection, scabies, inflammation and venereal diseases. The present study was to screen for antibacterial properties of different organic and aqueous crude extracts of leaves of *Tragia involucrata* is tested against *Escherichia coli, Proteus vulgaris* and *Staphylococcus aureus* by hole-plate diffusion method at different concentrations 50 mg/ml. The result revealed that ethyl acetate, methanol and water extract of leaf exhibited more activity than other extracts. The aqueous leaf extract was the most active against *P. vulgaris* and *S. aureus* and it was compared to chloramphenicol (30μg/disc) and streptomycin (30μg/disc). The effective extracts were determined the Minimum Inhibitory Concentration at 25, 75 and 50 mg/ml. The MIC was recorded in aqueous leaf extracts at 75 mg/ml. Nine different fractions were eluted from the leaves of *T. involucrata* through silica gel column (60x3.2 cm). The isolated compounds were tested against bacteria and showed antibacterial activity. The effective fractions were subjected to UV, NMR and GC-MS analysis and (hydrocarbon esters) confirmed by spectral data. The compounds such as vinyl hexylether, shellsol, 2,4-dimethayl hexane, 2-methylnonane and 2,6-dimethyl heptane, among the tested compounds shellsol inhibited very effectively the growth of *S. aureus*. Antibacterial property and its various isolated agent of this plant seem to have a scientific basis for the usage in traditional medicine. And also it provides a first line of defence against infection by acting as 'natural antibiotics.'

Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 06803 1PF.1

DISULFIDE BOND REORGANIZATION AND ALANINE ANALOGUES OF κ-HEFUTOXIN1: STRUCTURE-FUNCTION RELATIONSHIPS

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κ-Hefutoxin1 (κ-HfTx1), a novel class of short-chain scorpion toxin, is a 22-mer peptide cross linked by two disulfide bridges isolated from the venom of the scorpion *Heterometrus fulvipes*. Contrary to the classical fold of scorpion toxins comprising double or triple stranded β-sheet and a stretch of α-helix, κ-HfTx1 adopts a unique three dimensional fold of two parallel helices linked by two disulfide bridges. κ-HfTx1 was earlier reported a blocker of voltage-gated K^+-channels, Kv1.3 and Kv1.2. In the present study, two disulfide bond isomers of κ-HfTx1 were chemically synthesized, isomer1 (C4-C18/C8-C22) and 2 (C4-C8/C18-C22), to study the role of disulfide bond rearrangements in the biological function of the toxin. Solution NMR of isomer1 showed a few long range NOEs connecting the aromatic amino acid side-chain to the backbone protons. Few residues flanking the disulfide bonds in the peptide appear to be structurally constrained. Electrophysiological studies of isomer1 showed an IC50 of 60 mM on Kv1.2 unlike the native peptide (150 μM), while no significant change was observed on Kv1.3 currents. Isomer2 had no activity on the different potassium channels studied. Furthermore, alanine analogues of the two glutamate residues (E16A and E20A) were also found to have an increase in the binding affinity towards Kv1.2 channel. The structure-function relationships of native, disulfide and other analogues of κ-HfTx1 will be discussed.

Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 06804 1PF.1

GENE EXPRESSION ANALYSIS OF AFLATOXIN B1: INSIGHT INTO THE MECHANISM OF TOXICITY IN HUMAN LIVER CELLS

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Aflatoxins are hepatotoxins produced by some strains of fungus (*Aspergillus*), which are found mainly in improperly stored food and animal feeds. Aflatoxin B1 (AFB1) is the most prevalent aflatoxins, which is reported as carcinogen of the liver. However, the molecular mechanism underlying the pathogenesis in human liver has not been well documented. The aim of this study was to assess the effect of subpathological levels of AFB1 on the gene expression profile of human liver cells. Chang Liver Cells (CLC) was exposed to 10 μM or 15 μM of AFB1 for a period of 12, 24 and 48 hrs, respectively and the expression pattern was analysed by Affymetrix Human GeneChip U133 A. Analyses of the data demonstrated that 195 and 177 genes were up- and down-regulated, respectively with significant fold changes >=2. Most of these genes were found to be involved in signal transduction, cell-cycle regulation, gene transcription and apoptosis, which are closely related to hepato-carcinogenesis. Genes involved in liver metabolism of AFB1, like Cytochrome P450, were significantly reduced. Interestingly, some genes related to lipid metabolism, such as phospholipid transfer protein and low-density lipoprotein receptor-related protein 5, were greatly up regulated. This was consistent with the pathological observed by histochemical changes. Besides, the hallmark mutation at codon 249 of P53 gene, which was reported by previous studies, the expression level of P53 cellular tumor antigen gene was increased with time and concentration. In addition, some of G- protein coupled receptors, RAS gene family and MYC were also affected. Our results suggest that AFB1 may have a broad role in affecting cellular physiology through modulating gene expression.

Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 07302 1PF.1

DO INSECTICIDAL J-ATRACOTOXINS TARGET INSECT POTASSIUM CHANNELS?

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The Janus-faced atracotoxins (J-ACTXs) are a three-membered family of insect-selective excitatory neurotoxins containing 36-37 amino acids with four disulfide bonds (Wang et al 2000). Toxins from this family were first isolated from the venom of the female Blue Mountains funnel-web spider, *Hadronyche versuta*. These peptides contain an unusual 'CCPCCP' motif and show no homology with any sequences in the protein or DNA databases. Therefore, the primary sequence provides few clues as to the likely target for this family of novel insect-selective neurotoxins. A recombinant expression system for the prototypic family member, J-ACTX-Hv1c, was developed that allowed mapping of the toxin pharmacophore. This revealed five key residues (Ile2, Arg8, Pro9, Val29, Tyr31) as well as an unusual vicinal disulphide (Cys13-Cys14) critical for toxicity in House crickets (Maggio & King, 2002). The functionally critical Arg8 and Tyr31 residues of this toxin align extremely well with the Lys-Phe/Tyr diad conserved amongst structurally dissimilar K^+ channel toxins, suggesting a possible site of action for the J-ACTX family. In order to characterise the site of action of this toxin, whole-cell patch-clamp electrophysiology was employed using isolated dorsal unpaired median (DUM) neurons from the terminal abdominal ganglion of the adult cockroach *Periplaneta americana*. J-ACTX-Hv1c had no effect on the gating or kinetics of Na^+ currents at concentrations up to 1 mM. However, at the same concentrations, it reduced whole-cell K^+ currents (IK) by up to 56 ± 7% (n=5), blocking both the peak and late IK. Since cockroach DUM neurons express a variety of K^+ currents, including transient 'A'-type, delayed-rectifier, Na^+- and two Ca^{2+}-activated IK, J-ACTX-Hv1c is targeting one or more K^+ channel subtypes. At concentrations up to 1 mM, J-ACTX-Hv1c caused a 24 ± 2% (n=3) reduction in the delayed-rectifier IK recorded in the presence of 1 mM Cd^{2+}, 150 nM tetrodotoxin and 5 mM 4-aminopyridine. However this effect was abolished in the presence of charybdotoxin (30 nM) a Ca^{2+}-activated IK blocker. This indicates that the most likely target of J-ACTX-Hv1c is an insect Ca^{2+}-activated K^+ channel. Further experiments aim to verify this hypothesis by determining the dose-dependency of this block and confirming that J-ACTX-Hv1c fails to modulate other insect K^+ channel subtypes.

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Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 07303 1PF.1

DISCOVERY OF A NOVEL SODIUM CHANNEL NEUROTOXIN δ-MISSULENATOXIN-MB1A FROM THE VENOM OF THE EASTERN MOUSE SPIDER MISSULENA BRADLEYI

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Australian mouse spiders (Araneae: Mygalomorphae: Actinopodidae) are one of the oldest groups of spiders in the Australo-Papuan region. Importantly, the male Eastern mouse spider (*Missulena bradleyi*) can cause serious envenomation in humans. The symptoms of envenomation, including tachycardia, dyspnea and profuse sweating, are remarkably similar to those reported for Australian funnel-web spider envenomation despite the spiders belonging to phylogenetically distinct families. In order to identify the toxin responsible for this envenomation syndrome male *M. bradleyi* venom was fractionated using analytical C18 reverse-phase HPLC. Potential neurotoxic fractions were bioassayed using an isolated chick biventer cervicis nerve-muscle preparation. The major neurotoxic fraction caused an increase in resting tension, muscle fasciculations and a decrease in indirectly-stimulated twitch contractions. The responses to exogenously applied ACh or KCl were unaffected in the presence of the toxin, however all responses could be inhibited by tetrodotoxin (TTX). This indicates that the toxin targets presynaptic nerve structures resulting in spontaneous neurotransmitter release consistent with the signs of envenomation. Interestingly, these effects were neutralised by antivenom raised against the venom of the Sydney funnel-web spider *Atrax robustus*. Electrospray ionisation mass spectrometry determined a molecular weight of 4932±1 Da. Subsequent Edman degradation of pyridylethylated toxin revealed a 42-residue peptide with unusual N- and C-terminal cysteines and a cysteine triplet (Cys14-16). This toxin was highly homologous to a family of δ-atracotoxins (δ-ACTXs) from funnel-web spiders showing 88% homology and 78% identity with δ-ACTX-Hv1a from *Hadronyche versuta* including conservation of all eight cysteine residues. δ-ACTXs have been shown to slow Na^+ channel inactivation in a manner analogous to scorpion α-toxins by binding to neurotoxin site-3 on the Na^+ channel. Whole-cell patch-clamping of dorsal root ganglion (DRG) neurons revealed that the toxin caused a slowing of TTX-sensitive Na^+ channel inactivation, and a hyperpolarising shift in the threshold of activation, in a manner similar to δ-ACTXs. Given the high degree of homology to δ-ACTXs the toxin was named δ-missulenatoxin-Mb1a (δ-MSTX-Mb1a) using the nomenclature previously described for funnel-web spider toxins. This provides evidence of a highly conserved toxin that has not undergone any significant modification.

Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 09902 1PF.1

ARDISCRETIN A NOVEL ARTHROPOD-SELECTIVE TOXIN FROM TITYUS DISCREPANS SCORPION VENOM

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A new arthropod selective toxin was purified from the venom of the Venezuelan scorpion *Tityus discrepans*. The cDNA, amino acid sequence and biological activity is reported. The amino acid sequence of this peptide was completed by Edman degradation and mass spectrometry. Ardiscretin (arthropod *discrepans* toxin) is a single polypeptide composed by 61 amino acids with an amidated cysteine residue at the C-terminal end, linked by four disulfide bridges. The molecular mass experimentally determined was 7102.8 Da. Sequences comparison showed that ardiscretin is phylogenetically closest to insect-specific scorpion toxins. This peptide was shown to be specific for arthropod as assayed in crickets, hemiptera and other invertebrates such as crab and squid axon. When ardiscretin is applied on squid giant axon inhibited the Na^+-currents in an apparent irreversible manner, whose inhibitory effect is reached with a 30 mM toxin concentration. Like excitatory insect toxins, ardiscretin produced a small depolarization and induced repetitive firing. The effect of ardiscretin in squid axon resembles those of DDT [1,1'(p-chlorobenzyl) 2-tricloretane] in its ability to slow down action potential and inducing repetitive firing, and in that the concentrations applied to squid axon are rather high. This work was partially supported by FONACIT grant N°: S1-2001000908 GDS, and CONACyT Z-005 to LDP.

Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 10401 1PF.1

CORRELATING STRUCTURE, EVOLUTION AND K+ CHANNEL SPECIFICITY OF SCORPION TOXINS

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Virtually all living cells posses K^+ channels, whose biochemical, pharmacological, physiological, biophysical and structural characteristics are being elucidated, due in part to the use of specific short chain peptides isolated from scorpion venoms (KTxs). The KTxs modulate channel function by binding to the outer mouth of the channel, most of which do so by physically occluding the ion pathway, others by mechanisms still to be determined. In order to confirm the various possible surface areas of contact between ligands and channels more experiments need to be performed. Here, we describe a novel approach that could help in designing such experiments. It is based on a phylogenetic analysis of known primary structures of KTxs and correlations with their described functions. Our analysis indicates a good correlation between sequence and target specificity, inasmuch as that a comparison of the electrostatic surface potentials of known KTxs three-dimensional structures further sustain these correlations. On the basis of such analysis, KTxs seems to display three alternative functional epitopes, whose also defines target specificity: a functional dyad of residues situated at the beta sheet face (for the KCa1.1 and KCa3.1 affecting KTxs), an apamin-like binding motif at the alpha helix (for the KCa2.x affecting KTxs) or a hydrophobic patch involving residues at the beta turn region (for the Kv11.x affecting KTxs). These alternative epitopes has been already proposed on the basis of direct experimental evidence, however several KTxs (sub-families 9, 11, 14 and 18) do not fit any of the alternative epitopes mentioned. Although it is difficult to correlate two apparently independent evolutionary processes, it is tempting to speculate that there is a co-evolutionary force driven maturation of the KTxs versus K^+-channels interactions. Maybe, complementary analysis like the one we are presenting here, could guide the design of experimental protocols to prove the point.

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Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 10501 1PF.1

PHAIDOTOXIN: A NOVEL INSECT-TOXIN PURIFIED FROM THE VENOM OF THE MEXICAN SCORPION ANUROCTONUS PHAIODACTYLUS

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Mexico has a great diversity of scorpions, estimated to be over 200 different species, from which only the scorpions of the genus *Centruroides* are known to cause deadly accidents to humans. Here we describe the isolation and characterization of an insect specific toxin from the scorpion *Anuroctonus phaiodactylus*, collected in Baja California. Human accidents occurred in this area, by this scorpion, has not been reported as medically important. When this venom is injected into experimental mammals at doses of up to 0.1 mg/20 g mouse weight they do not show symptoms of intoxication, whereas venom of other *Centruroides* scorpions are already lethal at doses as little as 0.0004 mg/20 g mouse weight. This venom injected into insects (crickets) is quite toxic. By means of several chromatographic steps (Sephadex G-50 and HPLC) we have isolated a pure peptide named Phaidotoxin, that at doses of 10 mg/Kg cricket weight is deadly. The peptide has 72 amino acids with molecular mass of 7978.3 a.m.u. Its full amino acid sequence was determined by Edman degradation and mass spectrometry. Phaidotoxin has four disulfide bridges, however the spatial position of one of these disulfide bridges is completely different from all the other insect toxins know. It is between Cys63 and Cys71. The three remaining disulfides of the core of the molecule are similar to other scorpion insect toxins. Several genes coding for this peptide and isoforms were isolated by PCR using cDNA prepared from RNA and specific oligonucleotides. Comparative sequence analysis shows that Phaiodotoxin belong to the long-chain scorpion sub-family of peptides, possibly a Na^+-channel type of toxin, however if this specificity is confirmed by electrophysiological experiments (under way now in the laboratory) it is going to be the first example of a new class of insect specific toxins.

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Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 10601 1PF.1

TOXIC SUBSTANCE IN NORI (DRIED PURPLE LAVER PRODUCTS) IMPLICATED TO FOOD POISONING

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In a food poisoning case, four persons had some allergic-like symptoms such as inflamed and red rash on their face, mouth and belly after eating dried purple laver product (called nori). The causative nori was extracted and smeared on the arm-skin of five volunteers. Three out of five volunteers had slight allergic reaction after 5 to 30 min when they were exposed to sunlight. The level of food additives monosodium glutamate (MSG) and sulfur dioxide was found to be low. While the levels of the derivatives of chlorophyll: pheophorbide and pyropheophorbide measured by HPLC were 890 and 5460 ppm in the causative sample, respectively. Judging from the high dose of pyropheophorbide and pheophorbide and symptoms of patients and volunteers, the causative agents were identified as photosensitive compounds pyrophroborbide and pheophorbide.

Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 12501 1PF.1

EFFECT OF ANTIVENOM ON THE IN VITRO NEUROTOXIC AND MYOTOXIC ACTIONS OF MICROPECHIS IKAHEKA VENOM

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The New Guinean small eyed snake (*Micropechis ikaheka*), which is widely distributed throughout Papua New Guinea and several Indonesian Islands, has been implicated in many cases of severe envenomation (Warrell et al., 1996). The predominant clinical features of envenomation include neurotoxicity, myotoxicity, haemoglobinurea and uncoagulable blood (Hudson & Pomat, 1988; Warrell et al., 1996). Treatment of envenomation is empirical with no specific antivenom available. In the present study, we examined the in vitro effects of M. ikaheka venom in the chick biventer cervicis muscle preparation. Venom (3-10μg/ml) produced a concentration-dependent inhibition of indirect (0.2ms, 0.1Hz, supramaximal V) twitches. This effect appeared to be postsynaptic in origin as evidenced by the inhibition of responses to exogenous acetylcholine (1mM) and carbachol (20μM) but not KCl (40mM). The inhibition was significantly reversed by the addition of CSL Ltd polyvalent snake antivenom (5U/ml) or black snake Antivenom (5U/ml) when twitch response reached 90% of initial;t_{90}, 37.6 min ± 1.9min at 3μg/mL. Venom (10-50μg/ml) also produced myotoxicity as indicated by a slowly developing contracture and inhibition of direct (2ms, 0.1Hz, supramaximal V, in the presence of tubocurarine 10μM) twitches. Myotoxicity was confirmed by histological examination of tissues. This was prevented by the prior incubation of tissues with polyvalent snake antivenom (5U/ml). These results indicate that CSL Ltd Polyvalent Snake Antivenom would be useful in the treatment of envenomation by *Micropechis ikaheka*.

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Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 12602 1PF.1

SMOOTH MUSCLE ACTIVITY OF BOIGA VENOMS

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Boiga is the most widespread genus of the opisthoglyphous (rear-fanged) colubrid snake family. Previously, it has been shown that venoms from this genus can act postsynaptically at the skeletal muscle neuromuscular junction (1, 2). The current study examined the pharmacology of venom from *Boiga cynodon, Boiga dendrophila* and *Boiga irregularis* on some isolated smooth muscle preparations. Each venom (50 µg/ml) produced a contractile response in the guinea pig isolated ileum (2-3 cm segments) which was significantly inhibited by the non-selective muscarinic receptor antagonist, atropine (0.3 µM), but not by the H1-receptor antagonist mepyramine (0.3 mM). Venoms (50 µg/ml) displayed no contractile activity in epididymal or prostatic segments of the rat isolated vas deferens. Further, venoms (50 µg/ml) had no significant effect on the contractile response to noradrenaline (25 µM), in the epididymal segment, or the inhibitory response to clonidine (3 nM), in the electrically stimulated (0.3 ms, 0.2 Hz, 60 V (supramaximal voltage)) prostatic segment. HPLC and SDS PAGE analysis, as well as PLA2 and protein assays, indicated that the venoms are qualitatively similar. In conclusion, we have shown that these venoms contract gastrointestinal smooth muscle via muscarinic receptor activation. Further studies are required to determine whether this is a direct effect (i.e. due to the presence of a parasympathomimetic agent) or an indirect action (i.e. due to the release of endogenous acetylcholine).

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Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 13801 1PF.1

MOLECULAR MECHANISM OF HEMOLYSIS BY BACILLUS CEREUS SPHINGOMYELINASE

Kikuo Tsukamoto, Takashi Obama, Koji Morikawa, Masayoshi Imagawa and Hiroh Ikezawa

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Bacterial sphingomyelinases catalyzing the hydrolysis of sphingomyelin to ceramide and phosphorylcholine, are classified into Mg^{2+}-dependent neutral sphingomyelinase, and known to belong to hot-cold hemolysins. These enzyme act on sphingomyelin-rich erythrocytes of mammals such as ruminants by degradation of sphingomyelin in the erythrocyte membrane. After partial hydrolysis of sphingomyelin, hot-cold hemolysis occurs when the erythrocytes are cooled to 4^0C, due to phase transition of the membrane. In addition, *Bacillus cereus* sphingomyelinase exhibits potent "hot hemolysis", hemolysis without chilling the erythrocytes by further degradation of sphingomyelin. This enzyme is activated by Mg^{2+}, but inhibited by Ca^{2+} and EDTA. In the presence of Ca^{2+}, however, the enzyme is strongly adsorbed to the membranes of sphingomyelin-rich erythrocytes. Due to the peculiar properties, the hemolytic activity of this sphingomyelinase is much more potently activated in the coexistence of Ca^{2+} and Mg^{2+} than in the presence of Mg^{2+} alone.

We focused on the structure-function relationship of B. cereus sphingomyelinase especially involved in the catalytic and membrane-adsorbing sites. Therefore, we predicted the three-dimensional structure of the sphingomyelinase by a 3D-1D protein-fold recognition method, showing the primary sequence of this enzyme to exhibit a high compatibility with the X-ray structure of bovine DNase I. In our 3D model of the sphingomyelinase, several conserved residues among bacterial sphingomyelinases converge on the active-site pocket of the enzyme. On the basis of such structural characteristics, site-directed mutagenesis was applied to the conserved residues. Experimental results demonstrated that Asp100 must act as a ligand for Ca^{2+}, participating directly in adsorption of this enzyme to erythrocyte membrane. It is also showed that several hydrophobic residues may contribute to the adsorption step. Asp100 is surrounded with the protruding hydrophobic residues on the enzyme surface, suggesting Asp100 to construct the membrane-binding site with the hydrophobic residues. Also, we confirmed that His151 and His296 must be involved in the active center as acid-base catalysts, and Glu53 must well act as a ligand for essential Mg^{2+}. Asp195 proved to be essential for hemolytic activity since they seemed to interact with two catalytic His residues of the enzyme.

Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 13901 1PF.1

INHIBITION OF SNAKE VENOM ACTIVITIES BY MURINOGLOBULIN

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Local symptoms such as hemorrhage, necrosis, swelling and blister formation are major complications in the case of Viperidae snake envenomation. The efficacy of anti-venom administration against local symptoms has been reported to be poor because of rapid development of the damage and poor circulation around the bitten area. Most of the local and systemic complications following Viperidae snakebite are due to protease components of the venom. Especially, hemorrhagic metalloproteinase plays a crucial role in the development of local tissue damage, causing hemorrhage, edema, myonecrosis and skin damage. Rat murinoglobulin (MG) is a plasma glycoprotein belonging to the $\alpha_2$macroglobulin family. MG inhibits a wide variety of proteases, not only serine proteases but also metalloproteinases by steric hindrance. In order to obtain basic data on the effect of broad-spectrum protease inhibitor against local symptoms of Viperidae snake envenomation, inhibitory capacity of rat murinoglobulin on local hemorrhagic and edematogenic acitivities of venoms from five different genera, *Crotalus atrox, Bothrops jararaca, Lachesis muta muta, Trimeresurus flavoviridis* and *Echis carinatus shochureki*, were examined. Murinoglobulin, pre-incubated with the crude venoms, inhibited hemorrhagic activity of all five venoms to various extents. The hemorrhagic activity of Crotalus atrox was almost completely inhibited at the MG/venom ratio (w/w) of 20. The activity of *Bothrops jararaca, Lachesis muta muta* and *Trimeresurus flavoviridis* venoms was considerably inhibited at the ratio of 20, however some of the activity still remained even at the ratio of 40. Among the 5 venoms, *Echis carinatus sochureki* venom was quite resistant to murinoglobulin treatment and statistically significant inhibition was only found at the ratio of 40. Fibrinolytic and gelatinase activities were more susceptible to murinoglobulin inhibition. The treatment at the ratios of 10 and 20 almost completely inhibited respectively the fibrinolytic and the gelatinase activities of all the venoms. Murinoglobulin treatment also significantly inhibited the edematogenic activity of *L. muta muta, Trimeresurus flavoviridis* and *Echis carinatus sochureki*. The treatment of murinoglobulin at the ratio of 40 considerably suppressed the swelling up to 60 min after subcutaneous injection of *L. muta muta* and *Echis carinatus sochureki* venoms, and up to 30 min after *Trimeresurus flavoviridis* venom injection. Murinoglobulin is a potent inhibitor against local effects of multiple snake venoms in Viperidae family.

Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 15301 1PF.1

PHARMACOKINETICS OF SCORPION VENOMS IN ANIMAL MODELS STUDIED BY IMMUNOASSAYS: VARIABILITY IN ALPHA-TOXIN BIODISTRIBUTION.

Christiane Devaux[1], Mohamed Naceur Krifi[2], Besma Jouirou[1], Yannick Autier[1], Mohamed El Ayeb[2] and Hervé Rochat[1]

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Alpha-toxins from scorpion venoms act on the sodium channels of excitable cells and are responsible for most of the lethal effect of venom in mammals. Although many groups have studied the pharmacokinetics of crude and semi-purified venom from different scorpion species, there are no data about the behavior of the various individual alpha-toxins after envenomation. The objective of this study was to investigate the fate of Aah I, Aah II and Aah III, the highly active alpha-toxins from the dangerous *Androctonus australis hector* scorpion, following intravenous or subcutaneous injections into rabbits and mice. We used specific immunoassays to measure the concentration of each toxin in blood, urine and organs at various times after envenomation. Aah I, Aah II and Aah III could be detected in rabbit plasma within 5 min of a subcutaneous. injection of a sublethal dose of venom. Their concentration increased rapidly, peaking between 30 to 60 min and then slowly decreasing and becoming undetectable after 180 to 420 min. This rapid biodistribution was also observed in mice when Aah I and Aah II concentrations were measured in plasma, urine and organs. Thus, the toxins are rapidly absorbed, leading to high plasma concentration a short time after subcutaneous injection of venom followed by a progressive elimination. However, the concentration of Aah II was systematically lower than that of Aah I. In addition, in rabbits, Aah II appeared to be eliminated faster than Aah I or Aah III. This may be due to differences in the chemical and pharmacological properties of the different Aah alpha-toxins. Finally, pharmacokinetics of Bot I from the Buthus tunetanus occitanus scorpion venom were also presented. All our results support the early application of immunotherapy in the case of envenomation.

Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 15302 1PF.1

MONOCLONAL ANTIBODIES AND CONCEPTION OF RECOMBINANT ANTIBODIES POTENTIALLY USEFUL IN THE MANAGEMENT OF ENVENOMING.

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2- Muséum national d'Histoire naturelle, USM0505 Lerai, 57 rue Cuvier, 75005 Paris, France.

Scorpion stings cause widespread morbidity and mortality in all subtropical countries. When associated with symptomatic treatment, serotherapy is generally considered to be the only specific treatment of severe envenoming and is the most widely used approach. However, this treatment is not always efficient and improvements are required. First, we produced highly specific monoclonal antibodies against the main alpha-toxins responsible for the noxious effect that occurs in mammals stung by the dangerous *Androctonus australis hector* (Aah) scorpion. Secondly, in an attempt to explore new ways of making more effective therapeutic molecules, we designed recombinant antibody fragments that retain the biological properties of the most neutralizing monoclonal antibodies. The recombinant diabody 9C2 produced in E. coli was functional and had a high binding affinity for toxin I from Aah venom. This diabody showed a high level of thermal stability in serum and had protective activity when injected intraperitonally into mice experimentally envenomed with scorpion toxin Aah I. We designed and produced a bispecific diabody that mimicked two different binding sites: that of the anti-Aah I mAb (9C2) and that of the anti-Aah II mAb (4C1). This molecule retained the binding properties of the parent antibodies to the two main toxins of the Aah venom. We are currently testing the neutralizing properties of this bispecific diabody to the individual toxins and to the all toxicity of the venom. This kind of single molecule is very promising, as its properties can be further improved by molecular "tailoring". Further humanization could render this construction suitable for clinical applications.

Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 16101 1PF.1

SPATIAL STRUCTURE OF THE DEPRESSANT INSECT-SPECIFIC TOXIN LQHIT2 FROM LEIURUS QUINQUESTRIATUS HEBRAEUS.

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The depressant insect-specific toxin LqhIT2 purified from the venom of the Israeli yellow scorpion *Leiurus quinquestriatus hebraeus* was shown to exhibit a brief period of repetitive bursts of junction potentials, resembling the effect of excitatory toxins, followed by a block of neuromuscular transmission due to membrane depolarization when tested on insect neuromuscular preparation (Zlotkin et al 1991). Moreover, the toxin was shown to possess two noninteracting binding sites on insect neuronal membrane. The one binding site serves as a target for binding competition by the excitatory toxin. It is believed now that insect-specific toxin LqhIT2 can share properties of depressant as well as excitatory toxin. We used NMR spectroscopy to determine the spatial structure of the LqhIT2. The combination of NMR spatial structure analysis with sequence alignment methods allows us to explain how single toxin molecule may affect two different binding sites on the insect sodium channel.

Analysis of calculated LqhIT2 spatial structure shows the single type of backbone topology, which consists of antiparallel β-sheet and alpha-helix: residues 1-5 (I), 31-35 (III) and 42-45 (II) form three antiparallel beta-strands I-III and residues 18-29 form an alpha-helix, contacted with the IInd and IIIrd beta-strands. The average pairwise rmsd of 20 best structures calculated over the regions of the regular secondary structure (2-5, 18-29, 31-35 and 41-45) were equal to 0.39 ± 0.09 and 1.16 ± 0.28 Å for backbone and all heavy atoms, respectively. The C-terminal residues 46-61 possess the diversity of irregular conformations, while the 49-51 and 55-57 regions form helical turns in 11 and 15 of the 20 resulted structures, respectively. The obtained spatial structure of depressant insect-selective scorpion toxin LqhIT2 in combination with the sequence alignment methods allows us to outline the residues essential for its depressant and/or excitatory activities.

Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 16201 1PF.1

BITES BY POISONOUS PET ANIMALS IN JAPAN IMPORTED FROM FOREIGN COUNTRIES

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Recently in Japan, not only non poisonous snake but also poisonous snake, Gila monster and tarantula are imported as a pet. Especially, many dangerous poisonous snakes (Puff adder, Rhinoceros viper, Krait, Pigmy rattlesnake and coral snake) are dealt in at pet shops. And accidents by these poisonous animals sometime occur. Two cases of pigmy rattlesnake bite occurred in 1991. They have very poor knowledge of keeping and handling methods. Two green pit viper (*Trimeresurus alborabris* and *T. wagleri*) bites occurred but not serious.

On the other hand, there are some serious cases of Thai cobra (*Naja kaouthia*), Malayan krait (*Bungarus candidus*) and Chinese pit viper (*Agkistrodon brevicaudus*) bites. In 2001, the patient have been bitten by *B. candidus* while handling the snake which is a young small snake but the venom is highly toxic. The patient showed ptosis, ophthalmoplegia, ataxia and respiratory failure. Artificial respiration started 7 hours after the bite. The patient have been injected Chinese antivenom for *Bungarus multicinctus* venom with neostigmine and atropine. Supportive ventilation have been continued during 8 days. Three serious cases of Thai cobra bite occurred in 1969, 85 and 92 in Zoo The patients in 1969 and 85 developed respiratory paralysis and the patient in 1992 needed amputation of the finger. The patient by Chinese pit viper (*Agkistrodon brevicaudus*) in 1998 required ventilatory support for 60 days and hemodialysis for 12 days because of acute renal failure due to rhabdomyolysis.

There are problems in the treatment of the envenomation by foreign poisonous animals because no doctor have knowledge of their venom activities and experience of treatment, and no hospital in Japan have the antivenom except for the Japan Snake Institute. Therefore, the project group, supported by the Grant-in-Aid for Scientific Research from Ministry of Health, imported some antivenoms for these accidents but very rare.

Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 17302 1PF.1

ANTIGEN MICROARRAY: AN APPLICATION IN SNAKE VENOM STUDY

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A snake venom microarray was fabricated for the detection of snake antivenoms and the study of cross-reaction between snake venoms and antivenoms. This assay combines the sensitivity of fluorescence and high-throughput microspot technology. In this system, 22 venoms of snakes from Asia-Pacific region were spotted on the modified glass substrates in an array format. Normal rabbit antibody was included in the array as internal standards. Serum samples from venom immunized rabbits were incubated with the arrays. After antibodies in the samples were captured by antigens, the unbound proteins were washed away and the bound antivenoms were detected with Cy3 labeled anti-rabbit antibody. The signals were then visualized with microarray scanner and analyzed with QuantArray software. Experiments demonstrated that the fluorescent intensities were correlated well with antibody amounts in the standards. The reactivity of venom and antivenom was evaluated based on the intensity from corresponding spots. Antiserum against one crude venom cross-reacted with broad array of others, regardless their species. However the levels of cross-reactions were different. Higher reactivity was seen along the venoms of the same family and the highest cross-reaction was along snakes of the same genus. Microarray requires very little amount of sample and can provide both qualitative and quantitative results in one test. The concept should able to be extrapolated to develop a high-throughput venom microarray system for screening previously snake bitten subjects and possible alternative antivenoms.

Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 18303 1PF.1

FOLDING OF DM43, A METALLOPROTEINASE INHIBITOR FROM DIDELPHIS MARSUPIALIS

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Protein folding has evolved from a field of mere theoretical interest into an area of biological and medical relevance. It appears that folding intermediates, which have been linked to biological activity and to protein aggregation, are of paramount importance. Here, we have investigated the folding pathway of DM43, a homodimeric metalloproteinase inhibitor isolated from opossum serum. Denaturation of the protein induced by GdnHCl was monitored by extrinsic and intrinsic fluorescence spectroscopy. While unfolding of DM43 followed by tryptophan fluorescence only fits a cooperative two-state transition, extrinsic fluorescence studies reveal an intensity maximum at the midpoint of the unfolding transition and thus indicating an on-pathway intermediate state. Further analysis of DM43 by size exclusion chromatography support the view of a folding intermediate at distinct GdnHCl concentrations. In addition, by using hydrostatic pressure (up to 3.5 kbar) we were able to stabilize protein conformations different from the native and the fully unfolded state. Thus, it appears that the folding landscape of DM43 can be described by different local minima which might be important for stability and function of the protein.

Supported by CNPq, FAPERJ, FIOCRUZ and Howard Hughes Medical Institute.

Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 21901 1PF.1

EFFECTS OF ^{60}CO RADIATION ON BOTHROPSTOXIN-1 STRUCTURE.

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1-Laboratório de Química de Proteínas, Centro de Biologia Molecular, IPEN/CNEN, São Paulo, Brazil. 2- Toxinology and Aerobiology Division US Army Medical Research Institute for Infectious Diseases, Frederick, MD, USA. 3-Serviço de Radioproteção, IPEN/CNEN, São Paulo, Brazil. 4-Diretoria de Ensino, IPEN/CNEN, São Paulo, Brazil.

Gamma radiation is able to detoxify snake venoms and toxins without significantly affecting their immunogenic properties. This method has been successfully employed to attenuate toxins for antisera production without inducing toxic effects in animals undergoing immunization, However, the mechanism of attenuation is not fully understood and much work remains at the molecular level in order to further characterize the effects of radiation on these proteins. The present study was undertaken to evaluate structural modification following irradiation of bothropstoxin-1 (BthTx-1), a myotoxin from *Bothrops jararacussu*. It is believed that the functional form of the toxin is a homodimer with the binding affinity provided by electrostatic and hydrophobic interactions. Purified BthTx-1 was irradiated with 500, 1000 or 2000 Gy of ^{60}Co gamma radiation. The irradiated and native toxins were compared by mass spectrometry, circular dichroism (CD) and tryptophan fluorescence quenching. Results suggest that the monomer-monomer interactions are hydrophobic in nature. No significant difference were observed between the two forms of the toxins by CD spectral interpretation. However, significant losses of secondary structure could be observed when the native and irradiated BthTx-1 were compared after disulfide bond reduction. Fluorescence data indicates that the solvent accessibility of Trp 77 has been modified, which may explain the differences in quaternary structure.

Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 23001 1PF.1

A PHARMACOLOGICAL STUDY OF THE VENOM FROM PSEUDOCERASTES PERSICUS

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Pseudocerastes persicus is one of the most venomous snakes in the Iran plateau; however, little information on the pharmacological properties of the venom is available. Therefore, to extent our knowledge regarding this venom, the present study was carried out. The venom (0.1 - 3 mg/ml) produced dose-dependant blockade of the isolated guinea-pig ileum contractions induced by electrical field stimulation or by acetylcholine. 30 min after exposure of mouse phrenic nerve-diaphragm preparation to the venom (0.1-3 mg/ml), twitch responses to nerve stimulation were significantly reduced. In the chick biventer cervicis muscle, the venom (3 mg/ml) abolished nerve-mediated twitches (time to 90% inhibition: 70 ±10 min, n=9). In the chick biventer muscle, venom (1 mg/ml) significantly inhibited responses to acetylcholine (1 mM) and carbachol (20 mM), but not to KCl (40 mM), indicating activity at post-synaptic nicotinic receptors. Compare to the mouse hemidiphragm preparations, the chick biventer cervicis preparations displayed less sensitivity to the venom. The venom (3 mg/ml) decreased responses to direct muscle stimulation within 2 hours. This inhibitory effect of the venom was significantly reduced by prior incubation of the venom with manoalide, a phospholipase A2 inhibitor, indicating involvement of a phospholipase component. Further investigation is required to identify specific toxins with the above pharmacological effects.

Key words: *Pseudocerastes persicus*, Venom, Neuromuscular function

Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 23101 1PF.1

ANTI-VENOM POTENTIAL OF BUTANOLIC EXTRACT OF ECLIPTA PROSTRATA AGAINST MALAYAN PIT VIPER VENOM

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The butanolic and purified butanolic extracts of *Eclipta prostrata* were evaluated for their anti-venom potential. Inhibition of lethal, hemorrhagic, proteolytic, and phospholipase A_2 activities of *Calloselasma rhodostoma* (Malayan pit viper, MPV) venom by these extracts were determined. Demethylwedelolactone was identified as their major constituent. The butanolic extract, at 2.5mg/mouse, was able to completely neutralize the lethal activity of $2LD_{50}$ of MPV venom, but increasing the dose diminished the effect. The purified butanolic extract, at 1.5-4.5 mg/mouse, was able to neutralize the lethality of the venom at around 50-58%. Both extracts partially inhibited the hemorrhagic activity but displayed very low anti-phospholipase A_2 activity and did not inhibit proteolytic activity of MPV venom.

Poster Session 1PF.1 - Glass Foya - 1100-1140

ABSTRACT NUMBER 17303 5CR4

AN ANTIBODY MICROARRAY FOR THE DETECTION OF SNAKE VENOMS

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A snake venom antibody microarray was fabricated for simultaneous detection of species and quantitation of snake venoms. This assay combines the sensitivity of fluorescence and high-throughput microspot technology. In this system, species specific venom antibodies to four common snakes of Vietnam (*Trimeresurus popeorum, Calloselasma rhodostoma, Naja naja* and *Ophiophagus hannah*) and the four venoms were spotted on the modified glass substrates as the capture antibodies and internal standard antigens in an array format. Venom samples were then incubated with arrays. After antigens in the samples bind to their corresponding capture antibodies and unbound proteins are washed away, the bound venoms were detected with a mixture of species specific venom antibodies labeled with Cy3 or Cy5 (Two antibodies with Cy3 and two with Cy5). The signals were finally visualized with microarray scanner and analyzed with QuantArray software. Experiments demonstrate that the fluorescent intensity correlated well with venom amount in the standards. The species of venoms are indicated by position and colour of the spots which show positive signal. Quantitative analysis is done by comparison fluorescent intensity of positive spots with internal standards. Study on 88 serum sample of snake bite victims show that the assay is well correlated with conventional ELISA. However, microarray require very little amount of sample and can provide both qualitative and quantitative results in one test. The concept should able to be extended to develop a high-throughput antibody array system for venom detection.

TUESDAY September 16th

SCIENTIFIC PROGRAMME

Plenary Session 2AH - Hall C - 0900-1030

Session Chairperson: Neville Marsh

2AH1 Arthur M. Friedlander
ANTHRAX: CLINICAL FEATURES, PATHOGENESIS, PROPHYLAXIS AND TREATMENT

2AH2 Jay W. Fox
PRO-INFLAMMATORY EFFECTS OF JARARHAGIN ON FIBROBLASTS: INSIGHTS IN TOXIN-INDUCED INFLAMMATION USING GENE EXPRESSION ANALYSIS

Poster Session 2PF.2 - Glass Foya - 1100-1140

Poster Abstracts can be found at the end of Tuesday abstracts

Invited Lecture Session 2BH - Hall C - 1140-1305 - Bioterrorism

Session Chairperson: Art Friedlander

2BH1 Leonard A. Smith
BOTULISM

2BH2 Andrew Robertson
RISK MANAGEMENT AND BIOTERRORISM: WHAT IS THE REAL THREAT?

2BH3 Gary Phillips
THERAPEUTIC APPROACHES TO INHALED TOXINS

2BH4 Gareth D Griffiths
RETROSPECTIVE IDENTIFICATION OF RICIN EXPOSURE

Invited Lecture Session 2BM - Meeting Room 1&2 - 1140-1305

Session Chairperson: Usherwood

2BM1 Ray S. Norton
STRUCTURE AND MEMBRANE INTERACTIONS OF EQUINATOXIN II, A B-SANDWICH THAT FORMS OLIGOMERIC PORES IN MEMBRANES

2BM2 Yuri Ushkaryov
THE BLACK WIDOW SPIDER TOXINS, VERSATILE TOOLS TO STUDY NEUROSECRETION

2BM3 Anna M Moura-da-Silva
EVIDENCES FOR PARTICIPATION OF P-III SVMP DISINTEGRIN DOMAIN IN JARARHARAGIN INDUCED INFLAMMATION AND HEMORRHAGIC ACTIVITY

Oral Papers Session 2CH - Hall C - 1400-1530 - Ion Channel Toxins

Session Chairperson: David Adams

2CH1 Silke Kauferstein
SEARCHING FOR CONOTOXINS LEADS TO THE CHARACTERISATION OF A NEW POTASSIUM CHANNEL IN AN ANCESTRAL NERVOUS SYSTEM

2CH2 G. Corzo
SPIDER TOXINS THAT BIND TO THE SITE-4 OF THE INSECT SODIUM CHANNEL

2CH3 Michael Gurevitz
MOLECULAR BASIS OF SELECTIVITY OF SCORPION ALPHA-TOXINS FOR INSECTS

2CH4 HI Wilson
THE VENOM OF AUSTRALIAN URODACUS SCORPIONS (ARACHNIDAE: SCORPIONES: URODACIDAE) CONTAINS A NOVEL CLASS OF INSECT-SELECTIVE SODIUM CHANNEL-BLOCKING TOXINS

2CH5 Michael Gurevitz
A NOVEL 'OLD WORLD' BETA-TOXIN THAT RECOGNIZES INSECT AND MAMMALIAN SODIUM CHANNELS PROVIDES A CLUE ABOUT SCORPION TOXIN DIVERGENCE

2CH6 S Higuchi
A NOVEL PEPTIDE FROM THE ACEI/BPP-CNP PRECURSOR IN THE VENOM OF CROTALUS DURISSUS COLLILINEATUS

Oral Papers Session 2CR - Meeting Room 1&2 - 1400-1530 - Bioterrorism

Session Chairperson: Len Smith

2CM1 Mark A Poli
BLACK BIOCHEMISTRY: TOXINS AS WEAPONS OF BIOWARFARE AND BIOTERRORISM.

2CM2 Mark A Poli (contd)

2CM3 Mark A Poli (contd)

2CM4 S. Ashraf Ahmed
ENZYMATIC ACTIVITY OF AUTOCATALYTICALLY FRAGMENTED LIGHT CHAIN OF BOTULINUM A NEUROTOXIN

2CM5 A Shlosberg
MORTALITY IN A GROUP OF CATS PROBABLY CAUSED BY CLOSTRIDIUM BOTULINUM TYPE C TOXIN

2CM6 Frederic A. Meunier
GETTING MUSCLES MOVING AGAIN AFTER BOTULINUM TOXIN: NOVEL THERAPEUTIC CHALLENGES

Oral Papers Session 2CR - Meeting Room 3 - 1400-1530 - Biology & Evolution of Toxins

Session Chairperson: P Gopalakrishnakone

2CR1 M Lehvaslaiho
SWISS-PROT PROTEIN KNOWLEDGEBASE AND TOXINS

2CR2 W. Wüster
WHAT, IF ANYTHING, CAN VENOM MASS SPECTROMETRIC DATA TELL US ABOUT SNAKE PHYLOGENY?

2CR3 C. E. Pook
ASSESSMENT OF PHYLOGENETIC SIGNAL IN TOXIN DATA THROUGH COMPARISON WITH DNA SEQUENCE INFORMATION

2CR4 B.G.Fry
THE MOLECULAR EVOLUTION OF COLUBROIDEA SNAKE VENOMS

2CR5 Joanna Pawlak
CLONING AND EXPRESSION OF THE CDNA ENCODING A NOVEL THREE-FINGER TOXIN IN BOIGA DENDROPHILA (MANGROVE SNAKE) VENOM

2CR6 Cassian Bon
TRIMERUSURUS STEINEGERI VENOM PLASMINOGEN ACTIVATOR STRUCTURE, FUNCTION AND MODEL FOR THROMBOLYTIC AGENTS

Hypothetical - Hall C - 1600-1730

Session Facilitators: David Caldicott & Nick Edwards

Expert Panel: details released at this session

"Gamekeeper turned poacher - or - How this panel killed this Congress"

Bioterrorism is a real and current problem. As toxinologists we have a particular responsibility to consider issues surrounding our work and bioterrorism. This session has been designed to help illustrate the mechanisms of bioterrorism, using toxins, the problems that may be encountered and how we might deal with them. Though the topic is certainly not amusing, the purpose of this session is to promote learning and understanding. This is often best achieved through a degree of enjoyment. The facilitators and panel, hopefully with active participation and assistance from the audience, will try and achieve this, including the use of humor. Please be assured that none of those involved in organising this session see the issue of bioterrorism as humorous; any levity is in the interests of promoting ultimate understanding of the issues, as a learning tool. All matters to be raised by the facilitators are sourced from freely and readily available material, either published or on the internet. All participants are asked to avoid discussing "secret" information that might benefit potential terrorists.

TUESDAY September 16th

Plenary Lecture 2AH.1 - Hall C - 0900-0950

ABSTRACT NUMBER 04901 2AH1

ANTHRAX: CLINICAL FEATURES, PATHOGENESIS, PROPHYLAXIS AND TREATMENT

Arthur M. Friedlander, M.D.

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In 1990 during the Gulf War and again in 1998, for the first time in human history, a human population was vaccinated, not to prevent a naturally occurring disease, but rather against the threat of intentionally using a microorganism to cause disease. This occurred when the decision was made to vaccinate the U.S. Armed Forces against anthrax. The successful use of *Bacillus anthracis* as a bioterrorist weapon against civilians in the fall of 2001 has irrevocably altered our lives and the practice of medicine and public health. In this presentation, I will give an overview of the threat, the biology of the organism, the clinical disease, the pathogenesis and efforts to develop improved treatments and vaccines.

Plenary Lecture 2AH.2 - Hall C - 0950-1030

ABSTRACT NUMBER 01201 2AH2

PRO-INFLAMMATORY EFFECTS OF JARARHAGIN ON FIBROBLASTS: INSIGHTS IN TOXIN-INDUCED INFLAMMATION USING GENE EXPRESSION ANALYSIS

Paul G. Gallagher, Alyson Prorock, Solange M. T. Serrano, David R.D.G. Theakston, Aura S. Kamiguti, Gavin Laing, Cornelia Mauch, Paola Zignino, John D. Shannon, Junho Kim and **Jay W. Fox**

University of Virginia, Charlottesville, VA. USA (PG;AP;SMTS;JDS; JK:JWF), University of Liverpool and Tropical Research Institute, Liverpool, England (DRDGT;AK;GL), University of Cologne, Cologne, Germany (CM;PZ)

Many of the local effects of snake envenoming appear to be initiated immediately and subsequent administration of antivenin does not always effectively attenuate these local effects. One plausible explanation is that the time required for infiltration of the antivenin to the local site is such that local proteolytic degradation of tissues is already well underway by the time effective concentrations of antivenin are available at the site. We have been exploring additional mechanisms for local tissue destruction such as the induction of the host's pro-inflammatory system by snake venom metalloproteinases. Recently, we demonstrated that the PIII snake venom metalloproteinase jararhagin is capable of causing the up-regulation of the expression of the $\alpha_1\beta_2$ integrin, MMP1 and MT-1 MMP in human fibroblasts (Zigrino etl al. JBC 277:40528-35, 2002). We have now extended these experiments to determine the large scale effects of jararhagin on fibroblast gene expression using Affymetrix U95a GeneChip technology. The overall effect of jararhagin treatment of fibroblasts appears to be the initiation and persistence of the pro-inflammatory pathway via the up-regulation of relevant genes associated with the inflammatory response. This we believe could give rise to additional local tissue destruction that often accompanies Viperid envenoming. The genes involved in triggering the pro-inflammatory pathway will be described and a general mechanism for the development and persistence of inflammation in the host will be discussed.

Invited Lecture 2BH.2 - Hall C - 1140-1210

ABSTRACT NUMBER 06301 2BH1

BOTULISM

Leonard A. Smith

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Botulism is a paralytic disease caused by one of seven homologous neurotoxins produced by a common soil bacterium, *Clostridium botulinum*. The toxin exerts its action by arresting the release of the neurotransmitter, acetylcholine, at the neuromuscular junction causing flaccid paralysis. Clinical manifestations of the disease usually begin with cranial nerve dysfunction affecting the muscles of the head and neck (e.g., ptosis, blurred vision, diplopia, dry mouth and throat, dysphagia, and dysphonia). Cranial nerve palsies are followed by symmetrical descending flaccid paralysis, with generalized weakness and progression to respiratory failure. Symptoms begin as early as 12-36 hours after exposure, but may take several days depending on serotype and dose of toxin. Botulinum toxin (a CDC category A agent) constitutes a potential biological threat due to its extreme potency and lethality, the need for prolonged intensive care among survivors, worldwide accessibility of *C. botulinum* strains, and ease of large-scale production and transport. Development of vaccines and therapeutic intervention(s) that protect against botulism has been a focus of DOD efforts for over a decade. More recently, the DHHS has identified the area of botulinum neurotoxin as a national priority, and has initiatives underway through NIAID to support research and development activities that will lead to medical countermeasures against botulism. This presentation will review the current approaches to the development of vaccines and therapeutic interventions to botulism.

Disclaimer:
Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

Invited Lecture 2BH.2 - Hall C - 1210-1235

ABSTRACT NUMBER 03101 2BH.2

RISK MANAGEMENT AND BIOTERRORISM: WHAT IS THE REAL THREAT?

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Bioterrorism, the deliberate use of biological weapons by a terrorist group, has become a major concern for Australian medical, government and military agencies over the past five years. The various forms of media, from reputable newspapers and journals to novels and films, regularly portray such attacks. Despite these depictions, the true threat of bioterrorism in Australia remains uncertain. The dimensions of this threat, from capability to intent, need further exploration to enable risk management principles to be applied and to allow a realistic risk appreciation of the required response to be developed. In this presentation, the various determinants of risk and threat in a bioterrorism scenario will be examined. Once such a framework is developed, the suitability of planned responses can be examined and those initiatives that are most likely to be effective can be identified. Such a review will enable an honest appraisal of where Australia is in its capability, where it should be and where it needs to go. Whilst not a threat agent of major concern, microcystin, a toxin derived from blue-green algae, will be used as a case study in this presentation.

Invited Lecture 2BH.3 - Hall C - 1235-1250

ABSTRACT NUMBER 08301 2BH3

THERAPEUTIC APPROACHES TO INHALED TOXINS *

Gary Phillips, Simon Knight and Jane Holley

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Toxins produced by plants, animals and bacteria are among the most potent and lethal chemicals known to man. Several of these toxins are believed to form part of the biological warfare capabilities of many countries and in a conflict, deliberate exposure to toxins is likely to occur through the inhalation of disseminated aerosols.

The most potent of the toxins are the neurotoxins produced by the spore forming bacterium, *Clostridium botulinum*. To date seven serotypes have been identified, four of which, A, B, E and F are associated with disease in man. Another toxin of considerable interest is ricin. It has a long history of misuse and more recently is believed to have been prepared for potential acts of terrorism. Ricin is a plant-derived toxin found in the seeds of the plant *Ricinus communis*, which is grown and harvested commercially on a large scale through out the world. After castor oil extraction, ricin represents approximately 5% weight in the remaining waste mash. As many tonnes of castor oil beans are processed worldwide every year, this represents a vast reservoir of potential toxic material that could be available for misuse.

Current therapeutic approaches to toxin exposure are based upon the principle of neutralisation using antibodies conveyed either passively, as antitoxins or actively by the recipient of appropriate vaccine. For botulinum exposure, the horse derived anti toxins or the formalin inactivated pentavalent vaccine are available. However, in both cases, these therapeutic approaches are ineffective against exposure to two of the seven serotypes and anti toxin treatment is associated with undesirable side effects. For ricin exposure, there is currently no therapeutic approach or licensed vaccine. Treatment for exposure is symptomatic and supportive.

This presentation will highlight the current approaches undertaken at the Defence Science and Technology Laboratories, Porton Down on the development of a new generation of vaccines and antitoxins to the inhaled toxins, *Clostridium botulinum* and *Ricinus communis*.

Invited Lecture 2BH.4 - Hall C - 1250-1305

ABSTRACT NUMBER 08401 2BH4

RETROSPECTIVE IDENTIFICATION OF RICIN EXPOSURE

Gareth D Griffiths

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Ricin is a potent protein toxin produced by the seeds of the castor oil plant, *Ricinus communis*, which exists in a number of varieties growing in tropical and sub-tropical climates. Ricin is cytotoxic because it disrupts ribosomal infrastructure, inhibiting protein synthesis leading to cell death and ultimately, to the death of the whole organism.

Exposure to ricin would, most likely, be undetected and symptoms of intoxication typically develop several hours afterwards, depending upon the route of exposure and dose. Ricin has a long history of malevolent misuse, dating back to ancient times. More recently, the death of the Bulgarian journalist Georgi Markov in London in 1978, involved a minute but lethal quantity of a toxin, strongly believed to have been ricin, packaged into a small hollow sphere. This was propelled from an air gun device into Markov's thigh while he stood at a bus stop; he died three days later from an unknown cause. These days, a plethora of information on ricin is available on the www.

Retrospective identification of ricin poisoning in body tissues is a key requirement from a legal perspective and early identification could inform appropriate therapeutic intervention. However, this presents a particular challenge because of the low levels of material involved and its potentially widespread distribution in the body. The techniques for extraction of ricin from key target tissues and its identification and quantification using a bespoke sensitive ELISA will be discussed. The sensitivity of the indirect sandwich ELISA, using a conventional streptavidin/biotin detection system, is in the order of 0.2 ng/ml of sample extract. It is likely that more sensitive detection systems such as chemiluminescence would extend the boundaries of detection. Results of assays for quantification of ricin in rodent tissues will be presented.

Invited Lecture 2BM.1 - Hall C - 1140-1210

ABSTRACT NUMBER 07201 2BM1

STRUCTURE AND MEMBRANE INTERACTIONS OF EQUINATOXIN II, A β-SANDWICH THAT FORMS OLIGOMERIC PORES IN MEMBRANES

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Sea anemone cytolysins (actinoporins) are highly basic proteins of mass ~20 kDa that generate pores in membranes containing sphingomyelin (SM) by forming assemblies of 3-4 monomers in the membrane. The pores are permeable to small molecules and solutes and the resulting osmotic imbalance promotes cell lysis. The potency and properties of these cytolysins have prompted their evaluation as the toxic component of chimeric proteins targeted at tumour cells and human parasites. The actinoporins share no significant sequence identity with other pore-forming proteins, making it likely that they have a unique structure and mechanism of action.

Equinatoxin II (EqT II) is a 179-residue cytolysin from the Mediterranean anemone Actinia equina L. (1). The structure of EqT II, determined by NMR on 13C/15N-labelled protein, consists of two short helices packed against opposite faces of a beta-sandwich structure formed by two five-stranded beta-sheets (2). 15N relaxation data show uniform backbone dynamics, implying that EqtII in solution is relatively rigid, except at the N-terminus.

We have also investigated its interaction with micelles containing SM, an important constituent of membranes that are susceptible to lysis by this toxin. In addition, 2H and 31P solid-state NMR have been used to study the effect of EqT II on the structure and dynamics of bilayer lipids in multilamellar vesicles (3). The toxin appears to enhance slow motions in the membrane lipids and destabilize the membrane. This effect was greatly enhanced in SM-containing mixed lipid membranes compared with pure phosphatidylcholine bilayers. Further studies, including by 19F NMR, are directed at defining the membrane-binding surface of EqT II and conformational changes associated with lipid binding.

1. Macek, P. & Lebez, D. (1988) Toxicon 26, 441-451; Simpson, R.J., Reid, G.E., Moritz, R.L., Morton, C. & Norton, R.S. (1990) Eur J Biochem 190, 319-328.
2. Hinds, M.G., Zhang, W., Anderluh, G., Hansen, P.E. & Norton, R.S. (2002) J Mol Biol 315, 1219-1229; Zhang, W., Hinds, M.G., Anderluh, G., Hansen, P.E. & Norton, R.S. (2000) J Biomol NMR 18, 281-282.
3. Bonev, B.B., Lam, Y-H., Anderluh, G., Watts, A., Norton, R.S. & Separovic, F. (2003) Biophys J 84, 2382-2392.

Invited Lecture 2BM.2 - Hall C - 1210-1235

ABSTRACT NUMBER 06101 2BM2

THE BLACK WIDOW SPIDER TOXINS, VERSATILE TOOLS TO STUDY NEUROSECRETION

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The black widow spider venom contains several large protein toxins – latrotoxins – that are targeted selectively against different classes of animals: vertebrates, insects, and crustaceans. These toxins undergo proteolytic processing and activation in the lumen of the venom gland. The mature latrotoxins demonstrate functional structure conservation and contain multiple ankyrin repeats, which mediate toxin oligomerisation. All latrotoxins cause massive release of neurotransmitters from nerve terminals of respective animals after binding to specific neuronal receptors and by acting through several Ca^{2+}-dependent and -independent mechanisms based on a) pore formation and b) activation of receptors. Two major high-affinity receptors have been identified for α-latrotoxin, the venom component toxic to vertebrates: a single-transmembrane neurexin and a G protein-coupled latrophilin. The 3D structure and mutations of α-latrotoxin have allowed dissecting some of its actions: The wild type toxin assembles into tetramers, with a central channel, that can insert into lipid membranes and form pores. In contrast, a mutant recombinant α-latrotoxin ($LTXN^{4C}$) is unable to tetramerise and, therefore, insert into membranes or make pores. $LTXN^{4C}$ still binds the α-latrotoxin receptors and causes strong transmitter exocytosis in synaptosomes and chromaffin cells; it greatly enhances the frequency of miniature postsynaptic events recorded from CA3 pyramidal neurons and neuromuscular junctions. The effect of $LTXN^{4C}$ is reversible and requires extracellular Ca^{2+}, but is not attenuated by La^{3+} or other blockers of α-latrotoxin pores and/or Ca^{2+} channels. Acting via receptors, $LTXN^{4C}$ stimulates phospholipase C and intracellular Ca^{2+} stores and increases presynaptic free Ca^{2+} concentration. In contrast to wild type α-latrotoxin, $LTXN^{4C}$ potentiates evoked synaptic currents. Neurexins are not critical for these receptor-mediated effects. We suggest that mutant $LTXN^{4C}$ activates presynaptic latrophilin and stimulates Ca^{2+} release from intracellular stores, leading to the enhancement of synaptic vesicle exocytosis. These multidisciplinary studies have led to important insights into the mechanisms of α-latrotoxin action and the receptor signalling.

Invited Lecture 2BM.3 - Hall C - 1235-1305

ABSTRACT NUMBER 06901 2BM3

EVIDENCE FOR PARTICIPATION OF P-III SVMP DISINTEGRIN DOMAIN IN JARARHARAGIN INDUCED INFLAMMATION AND HEMORRHAGIC ACTIVITY

I. Tanjoni [1], M. S. Della-Casa [1], P. B. Clissa[1], L. Bento [1], M. L. Ferreira [1], D. Butera [1], I. Fernandes [1], J. M. Gutierrez [4] and **A. M. Moura-da-Silva** [1,3]

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Snake venom metalloproteinases (SVMPs) are synthesized as zymogens and undergo proteolytic processing resulting in a variety of multifunctional proteins. Jararhagin, a P-III SVMP isolated from the venom of *Bothrops jararaca*, is found in crude venom as two forms: full-length jararhagin and jararhagin-C, a proteolytically processed form of jararhagin that lacks catalytic domain and is comprised of the disintegrin-like and cysteine-rich domains of jararhagin. Full-length jararhagin shows MMP-like activity correlated to the hemorrhagic property of the toxin. Jararhagin-C presents disintegrin activity inhibiting $\alpha 2\beta 1$ integrin binding to collagen. Moreover, jararhagin presents structural and functional similarity with TACE (ADAM 17 - TNF-α convertase), interferes with neutrophil migration in extra-vascular tissues and also induces expression of citokines. These effects are not entirely dependent on jararhagin catalytic activity and imply a possible binding to inflammatory cells, suggesting the participation of jararhagin disintegrin domain. This hypothesis has been approached by testing biological activities of jararhagin-C obtained in the native and recombinant forms, and neutralizing ability of monoclonal antibodies specific to jararhagin-C. Jararhagin-C showed a distinct effect on expression of citokines and leukocyte activity in experiments carried out using experimental animal models. Besides that, MAJar3, a monoclonal antibody that recognize the C-terminus of the disintegrin domain, was able to neutralize jararhagin hemorrhagic activity and inhibited its binding to collagen. However, MAJar3 failed in neutralizing jararhagin catalytic activity on peptide substrates. Taken together, these data suggest that disintegrin domain is a key component in SVMP molecules both by binding to extracellular matrix and consequently modulating the catalytic activity and also by activating endothelial or inflammatory cells probably by agonist interaction to its integrin receptor.

Support: FAPESP, CNPq and Fundação Butantan

Ion Channel Toxins - Hall C - 1400-1415

ABSTRACT NUMBER 19201 2CH1

SEARCHING FOR CONOTOXINS LEADS TO THE CHARACTERISATION OF A NEW POTASSIUM CHANNEL IN AN ANCESTRAL NERVOUS SYSTEM

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One of the phylogenetically oldest nervous systems is found in *Hydra vulgaris*, a fresh water polyp. To detect voltage gated potassium channels in this nervous system, in particular those which are involved in muscular contraction, venoms from various cone snails were used, because these venoms provide a vast library of highly active peptides which interact selectively with key elements of the nervous system.

One fraction from the venom of *Conus virgo* was identified, comprising a peptide that causes a dramatic, but reversible contraction of the polyp's body column. The peptide was further characterized to be a specific inhibitor of vertebrate potassium channels of the Kv 1.1 and Kv 1.3 type. First PCR screening of cDNA from Hydra vulgaris reveals a corresponding K^+-channel fragment. Using 3´ race and the generacer methode the entire potassium channel gene was identified and the nucleotide sequence elucidated. Sequence aligment of the deduced amino acid sequence with various voltage gated K^+- channels and the hydropathic profile of the *Hydra vulgaris* potassium channel reveals the same overall structure (S1-S6) known from other voltage gated K^+-channels. In situ- hybridisation indicated that the channel is expressed exclusively in the sensory nerve cells of the polyp's head. The sequence of the potassium channel from *Hydra vulgaris* shows considerable homology to the Kv type potassium channel particular of more advanced animal phyla, suggesting that the potassium channel from Hydra vulgaris represents probably an ancestral ionic-channel.

Ion Channel Toxins - Hall C - 1415-1430

ABSTRACT NUMBER 14101 2CH2

SPIDER TOXINS THAT BIND TO THE SITE-4 OF THE INSECT SODIUM CHANNEL

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The crude venom of the spider *Paracoelotes luctuosus* (Araneae: Agelenidae) was analyzed for its toxicity against larvae of the crop pest *Spodoptera litura* (Lepidoptera: Noctuidae) using a microinjection bioassay technique. Four novel insecticidal toxins, named palutoxins 1 to 4 (PaluIT 1 to 4), were isolated and purified by reversed-phase and cation-exchange chromatography. The palutoxins are 36 to 37 amino acid homologous peptides reticulated by four disulfide bridges. The biological activity of PaluI, the most insecticidal peptide, was not significantly different ($P<0.05$) from that of AaIT, LqhaIT and LqhIT2, the most active scorpion toxins used in the development of recombinant baculoviruses. Competitive binding assays using a high affinity 125I-labelled scorpion peptide demonstrated the specific binding of palutoxins to the site-4 of the insect sodium channel. Alanine scanning mutagenesis of the paluIT2 showed that all basic residues are important for binding to the insect sodium channel. Moreover, two aromatic residues Trp12 and Tyr30 are crutial for receptor binding and insecticidal activity, and a mutation at Ser27Ala of paluIT2 had an effect on the correct folding of paluIT2 that caused the loss of binding and insecticidal activity. Palutoxins are the first spider toxins reported to bind to the site-4 of the insect sodium channel.

Ion Channel Toxins - Hall C - 1430-1445

ABSTRACT NUMBER 01702 2CH3

MOLECULAR BASIS OF SELECTIVITY OF SCORPION ALPHA-TOXINS FOR INSECTS

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Scorpion alpha-toxins are similar in mode of action and three-dimensional structure, but differ considerably in selectivity for various sodium channels (NaChs). To resolve the structural basis of this selectivity we compared two alpha-toxins that vary substantially in preference for insect versus mammalian NaChs. We have first determined by NMR the 3-D structure of the most potent anti-insect alpha-toxin known, LqhaIT (from *Leiurus quinquestriatus hebraeus*) and found that its alpha/beta-core (alpha helix packed against 3 anti-parallel beta-strands) is similar to that of other alpha-toxins. Then we employed an efficient system for toxin production and identified by mutagenesis its functional surface. Amino acid residues important for activity on insect NaChs appear in two clusters on the toxin surface. Phe17 and Arg18 of the loop preceding the alpha-helix, and Trp38 and Asn44 on the boundaries of the second and third beta-strands form the first cluster ("FRWN cluster"). The second cluster, formed by the C-tail and the 5-residue turn (residues 8-12), includes Tyr10, Ile57, Arg58, Val59, and Lys62 ("C-tail cluster"). In order to confirm the roll of these residues in the specific recognition of insect NaChs, we have produced a chimeric toxin, in which both clusters were constructed on the scaffold of Aah2, an anti-mammalian alpha-toxin that is poorly toxic to insects. The chimeric toxin, Aah2-{LqhaIT(face)}, has gained high toxicity to insects, as demonstrated upon injection to blowfly larvae, and in competition binding assays on cockroach neuronal preparations. We have determined the X-ray structure of the chimeric toxin at 1.3Å resolution. The most prominent deviation from the original Aah2 structure was observed at the C-tail region (positions 58-64), which adopted a conformation resembling that of LqhaIT. Construction of either the "FRWN cluster" or the C-tail on the scaffold of Aah2 did not improve the toxicity to insects. However, the spatial disposition of the C-tail determined by its interaction with the five-residue turn seems to be most critical for activity on insects. These results demonstrate that engineering of scorpion toxins affecting NaChs has become imminent.

Ion Channel Toxins - Hall C - 1445-1500

ABSTRACT NUMBER 07305 2CH4

THE VENOM OF AUSTRALIAN URODACUS SCORPIONS (ARACHNIDAE: SCORPIONES: URODACIDAE) CONTAINS A NOVEL CLASS OF INSECT-SELECTIVE SODIUM CHANNEL-BLOCKING TOXINS

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The Urodacidae are an endemic family of Australian scorpions containing twenty species within one genus, and are closely related to the Scorpionidae (Prendini, 2000). As they are not medically important, little is currently known of their venom composition or toxicity. This project aimed to identify and isolate insect-selective toxins for possible future application in insect pest control. The venom from two distinct species (*Urodacus manicatus* and *Urodacus hoplurus*) was collected and fractionated by several steps of size exclusion chromatography and reverse-phase HPLC. An aqueous/acetonitrile buffer system, acidified with 0.1% trifluoroacetic acid, was employed to enhance the selection of highly stable proteins. Purified peptide fractions had masses in the range 1.6 to 4.2 kDa as determined by electrospray ionisation mass spectrometry. Fractions were screened for toxicity at high concentrations in insects and vertebrates by injection into crickets (1-2 nmol/g) or by application to isolated chick biventer-cervicis nerve-muscle preparations (1-3 μM) respectively. Several toxic fractions were identified, showing either exclusive or partial selectivity against House crickets (*Acheta domesticus*) by direct lateroventral injection. Signs of toxicity included initial tremor, followed by ataxia and paralysis. With higher doses at or above the LD_{50}, death usually occurred within 24 hours. Many buthid scorpion toxins with this symptomatology target voltage-gated Na+ channels. Accordingly, patch clamp electrophysiology was conducted on acutely-isolated neurones from the terminal abdominal ganglion of adult American cockroach (*Periplaneta americana*) using voltage clamp techniques to investigate their effects on Na^+ channel gating and kinetics. This revealed that the predominant effect in these neurones was a reversible block of the peak INa, without any evidence of a shift in the voltage-dependence of Na^+ channel activation or inactivation. Unlike buthid Na^+ channels toxins that slow the rate of channel inactivation (α-, α-insect and α-like toxins; mass ~6.5-7.5 kDa) or shift the voltage dependence of activation to more hyperpolarized potentials, (β-toxins; mass ~6.0-7.0 kDa), these insect-selective Na^+ channel-blocking toxins have masses in the range of 2-4 kDa. This is supportive of a novel class of Na^+ channel toxins. Ongoing attempts at primary sequence elucidation are proving difficult, but should confirm the novel nature of these toxins.

Prendini L (2000) Cladistics 16: 1-78.

Ion Channel Toxins - Hall C - 1500-1515

ABSTRACT NUMBER 01703 2CH5

A NOVEL 'OLD WORLD' BETA-TOXIN THAT RECOGNIZES INSECT AND MAMMALIAN SODIUM CHANNELS PROVIDES A CLUE ABOUT SCORPION TOXIN DIVERGENCE

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Scorpion toxins that affect sodium channel (NaCh) gating in excitable cells are divided into alpha- and beta-classes. Whereas alpha-toxins have been found in scorpions throughout the world, beta-toxins, which affect mammals, have been assigned thus far to 'New World' scorpions, and anti-insect selective beta-toxins (depressant and excitatory) have been described only in the 'Old World'. This distribution suggested that diversification of beta-toxins into distinct pharmacological groups occurred after the separation of the continents 150 million years ago. We have characterized a unique toxin, Lqh-beta1, in the 'Old World' scorpion, *Leiurus quinquestriatus hebraeus*, that resembles in sequence and activity both 'New World' beta-toxins as well as 'Old World' depressant toxins. Lqh-beta1 competes with apparent high affinity with anti-insect and anti-mammalian beta-toxins for binding to cockroach and rat brain synaptosomes, respectively. Surprisingly, Lqh-beta1 competes also with an anti-mammalian alpha-toxin on binding to rat brain NaChs. Analysis of Lqh-beta1 effects on rat brain and Drosophila Para NaChs expressed in *Xenopus* oocytes revealed a shift in the voltage dependence of activation to more negative membrane potentials and reduction in sodium peak currents in a manner typifying beta-toxin activity. Moreover, Lqh-beta1 resembles beta-toxins by its weak effect on cardiac NaChs unlike its marked effect on the rat brain and skeletal muscle NaChs. These features suggest that Lqh-beta1 may represent an ancestral beta-toxin group in the 'Old World' that gave rise, after the separation of the continents, to depressant toxins in the 'Old World', and to various beta-toxin subgroups in the 'New World'.

Ion Channel Toxins - Hall C - 1515-1530

ABSTRACT NUMBER 15701 2CH6

A NOVEL PEPTIDE FROM THE ACEI/BPP-CNP PRECURSOR IN THE VENOM OF CROTALUS DURISSUS COLLILINEATUS

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In various crotaline venoms (e.g. *Bothrops jararaca*), angiotensin converting enzyme inhibitors (ACEIs, also known as bradykinin potentiating peptides (BPPs)) are products of a gene coding for an ACEI/BPP-CNP precursor (1). Sequencing of the cDNA clone showed that six different ACEIs/BPPs were located in a cluster at the N-terminus, and an additional sequence for C-type natriuretic peptide (CNP) was unexpectedly found at the C-terminus. Homologous genes for the ACEI/BPP-CNP precursor suggest that most crotaline venoms contain both ACEIs/BPPs and CNP (2). The sequence of ACEIs/BPPs is separated from the CNP sequence by a long spacer sequence. Until the present work, there was no evidence that this spacer actually coded any expressed peptides. Aird previously isolated a peptide of 11 residues (TPPAGPDVGPR) from *Crotalus v. viridis* venom (unpublished). In the present study, analysis of the cDNA clone from *Crotalus durissus collilineatus* revealed a nearly identical sequence in the ACEI/BPP-CNP spacer. Fractionation of the crude venom by reversed phase HPLC (C18), and analysis of the fractions by mass spectrometry indicated a component of 1020.5 Da. Amino acids sequencing by MS/MS method confirmed that *Crotalus durissus collilineatus* venom contains the peptide TPPAGPDGGPR. Its high proline content and paired proline residues are typical of venom hypotensive peptides, although it lacks the usual N-terminal pyroglutamate. The pharmacological activity of this peptide has not yet been determined; however, its occurrence in the venoms of two dissimilar species suggests that its presence is not accidental.

(1) Murayama, N. et al, PNAS 1997, 94, 1189-1193; (2) Higuchi, S. et al, Immunopharmacology 1999, 44, 129-135.

Bioterrorism - Meeting Room 1&2 - 1400-1445

ABSTRACT NUMBER 11602 2CM1-3

BLACK BIOCHEMISTRY: TOXINS AS WEAPONS OF BIOWARFARE AND BIOTERRORISM.

Mark A Poli

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The threat of bioterrorism or biological warfare by terrorist groups and rogue states is very real. Potential biological weapons include natural toxins as well as replicating organisms such as bacterial and viral agents. While the threat needs to be continuously evaluated, the description of biological agents as 'the ultimate terrorist weapon' is likely overstated, at least for toxins. The realistic number of potential toxin weapons is relatively low due to the limitations stemming from potency, stability, and availability. Effective weaponization and dissemination techniques are not trivial. In addition, countermeasures such as vaccines, diagnostics, and therapeutics confer significant protection. Although gaps exist in our knowledge base, research programs are in place to address these needs.

Disclaimer: Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the US Army.

Bioterrorism - Meeting Room 1&2 - 1445-1500

ABSTRACT NUMBER 20001 2CM4

ENZYMATIC ACTIVITY OF AUTOCATALYTICALLY FRAGMENTED LIGHT CHAIN OF BOTULINUM A NEUROTOXIN

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The light chain of botulinum A neurotoxin undergoes autocatalytic fragmentation that is accelerated by presence of the metal cofactor, zinc. The autocatalytic reaction is dependent on light-chain concentration, indicating that it is an intermolecular reaction. Fragmented light chain obtained in the absence of added zinc retained 100% of its original catalytic activity against a SNAP-25-derived synthetic peptide substrate. Fragmented light chain obtained in the presence of zinc chloride retained 35% of its original catalytic activity. On the other hand, when incubated in the presence of glycerol, the light chain did not display autocatalysis and retained 100% of the original activity. Substrate Km did not change appreciably by autocatalysis. These results suggest that the activity loss by incubation with zinc was not a direct consequence of autocatalysis and that the environment of the active site was not affected significantly by the fragmentation. The optimum pH 4.2-4.6 for autocatalysis was different than that (pH 7.3) for intrinsic catalytic activity. Inhibition of autocatalysis at low pH by a competitive inhibitor of catalytic activity rules out the presence of a contaminating protease but suggests a rate-limiting step of low pH-induced conformational change suitable for autocatalysis.

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Bioterrorism - Meeting Room 1&2 - 1500-1515

ABSTRACT NUMBER 09401 2CM5

MORTALITY IN A GROUP OF CATS PROBABLY CAUSED BY CLOSTRIDIUM BOTULINUM TYPE C TOXIN

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Botulism, the toxicosis caused by *Clostridium botulinum* toxins, is an often fatal disease of higher animals. Human botulism has been caused by toxin types A, B, E and F. The most common species affected in Israel is the bovine, by toxins C, D and rarely B. Wildlife botulism is mainly recorded in birds in wetlands in North America, where type C is most often involved. The means of exposure to the microorganism and/or its toxin is mainly decaying plant or animal tissues. It is therefore not surprising that carnivores have evolved a certain resistance to the action of the toxin, by mechanisms as yet largely unknown. There are therefore only rare reports of botulism in carnivores, and none in carrion-eaters. There have been several reports of type C botulism in dogs, sometimes fatal. There are no reports of natural botulism in cats, although cats are susceptible, being used as preferred research models for botulism.

An adult white pelican (*Pelecanus onocrotalus*) was found dead and was taken for studying the skeleton (Day 0). Muscle tissue (3-4 kg) of the pelican was offered to a group of 8 "back-yard" cats, who eagerly ingested all the meat. On the following day (Day 3), all the cats were mildly depressed and anorexic. One cat was recumbent and showed a flaccid paralysis, initially evident in the hind-limbs, and dyspnea. On Day 4, another cat showed similar signs, progressing to quadrapesis, and 3 more were affected to a lesser degree. On Day 5 the 2 very sick cats died, and all the other 6 cats showed mild to severe signs. On the night of Day 5-6, 2 more cats died. On Day 6, 5 cats were much improved, and the remaining sick cat was taken to the Veterinary Hospital, with muscle flaccidity, general poor reflex responses and hypothermia. After symptomatic treatment the cat was released on Day 8. Causes of toxicosis and infectious disease were tested and negated. *C. botulinum* type C bacteria were found in the stomach of one cat and in the pelican muscle. Very high levels of *C. botulinum* type C toxin were found in muscle tissue of the pelican taken off the skeletan. Two cats in the same group that were not in the yard when the meat was fed remained healthy throughout.

Circumstantial evidence of ingestion of the muscle found to contain very high levels of *C. botulinum* type C toxin, in the absence of any other plausible cause, brings us to the diagnosis of probable botulism, the first natural case reported.

Bioterrorism - Meeting Room 1&2 - 1515-1530

ABSTRACT NUMBER 14502 2CM6

GETTING MUSCLES MOVING AGAIN AFTER BOTULINUM TOXIN: NOVEL THERAPEUTIC CHALLENGES

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The use of botulinum neurotoxins in medicine for the treatment of muscle hyperactivity and spasticity disorders has been remarkably successful due to the toxins abilities to elicit prolonged localized paralysis, and the rarity of serious adverse effects. Botulinum toxins are however the most deadly protein toxins known and are part of the sinister arsenal of biological weapons. Although not very effective as an aerosol, it is alarming that few therapeutic treatments are available.

Paradoxically, the intoxicated motoneuron does not die. It reacts by emanating a sprouting network known to implement new functional synapses, leading to resumption of neurotransmission. Recent studies have highlighted ways of accelerating this natural recovery process to successfully overcome paralysis. Developing new therapeutic strategies and treatments for botulism will require more research into the molecular understanding of this "naturally-occurring" recovery process.

Biology & Evolution of Toxins - Meeting Room 3 - 1400-1415

ABSTRACT NUMBER 11901 2CR1

SWISS-PROT PROTEIN KNOWLEDGEBASE AND TOXINS

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Swiss-Prot Protein Knowledgebase (http://www.ebi.ac.uk/swissprot/) is a curated protein sequence database, which strives to provide high-quality annotation, a high level of integration with other databases and a minimal level of redundancy. It is maintained collaboratively by the Swiss Institute of Bioinformatics (SIB) and the European Bioinformatics Institute (EBI). Swiss-Prot is accompanied by TrEMBL, a computer-annotated protein sequence database, which can be considered a preliminary section of Swiss-Prot. TrEMBL contains translations of all coding sequences present in the DDBJ/EMBL/GenBank Nucleotide Sequence Database, and protein sequences that have been extracted from the literature or submitted to Swiss-Prot, which have not yet been integrated into Swiss-Prot. SPIN (http://www.ebi.ac.uk/submissions/) is a brand new web-based tool for submitting directly sequenced protein sequences and their biological annotations to the Swiss-Prot Protein Knowledgebase. SPIN allows fast submissions of single, multiple or large numbers of sequences to the database.

Swiss-Prot Release 41.11 (06-Jun-2003) contains 127863 entries, including 1175 toxins. Swiss-Prot's Tox-Prot project (http://ca.expasy.org/sprot/tox-prot/) aims to annotate systematically all proteins which act as toxins and are produced by venomous and poisonous animals. Each protein is annotated according to the quality standards of Swiss-Prot. The user is provided with far more than a simple collection of protein sequences, but rather a critical view of what is known or postulated about each protein. The entries consist of a wealth of information, such as function description, domains and sites, post-translational modifications, similarities to other proteins, as well as taxonomic data and citations. We strongly encourage contributions from the community.

Biology & Evolution of Toxins - Meeting Room 3 - 1415-1430

ABSTRACT NUMBER 20502 1CR2

WHAT, IF ANYTHING, CAN VENOM MASS SPECTROMETRIC DATA TELL US ABOUT SNAKE PHYLOGENY?

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Mass spectrometric methods, particularly LC/MS (liquid chromatography-mass spectrometry), are an important new tool for the investigation of snake venoms. The increased precision of these methods, compared to older approaches such as electrophoresis, has revived the interest in the use of venom-based data for the elucidation of snake taxonomy. Here we test the utility of LC/MS data for the recovery of snake phylogeny on three genera of Australasian elapids (blacksnakes – *Pseudechis;* death adders – *Acanthophis*; taipans – *Oxyuranus*), and compare these markers with a phylogeny derived from mitochondrial DNA sequences. Phylogenetic trees generated from LC/MS data are compared with trees generated from mtDNA sequences, and venom similarity is compared with genetic distance.

Although the LC/MS data contain a significant phylogenetic signal, the phylogenetic resolution achieved through the use of these markers is poor, and often incongruent with the mtDNA tree. Moreover, statistical support for most nodes was weak in the LC/MS data, which did not have the statistical power to reject alternative phylogenies, unlike the mtDNA data.

Similarly, the association between genetic distance and venom dissimilarity is low in all cases. The only generalization that can be made is that where two venoms do share a high proportion of molecular masses, they normally belong to phylogenetically closely related taxa.

We conclude that, while LC/MS remains the tool of choice for the analysis of the diversity of venom components in snakes, the resulting data have very limited potential as a taxonomic tool, and we therefore discourage taxonomic inferences based solely on these markers.

Biology & Evolution of Toxins - Meeting Room 3 - 1430-1445

ABSTRACT NUMBER 20601 2CR3

ASSESSMENT OF PHYLOGENETIC SIGNAL IN TOXIN DATA THROUGH COMPARISON WITH DNA SEQUENCE INFORMATION

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Toxinological studies using approaches such as electrophoresis and immunological cross-reactivity have long been used in attempts to resolve issues of snake phylogeny, but with mixed success. Newer venom-based data, such as toxin amino acid sequences and mass-spectrometric data provide a potentially more information-rich source of data for the elucidation of snake phylogeny. The genus *Dendroaspis* (mambas) presents an ideal model for testing the value of such toxin data. *Dendroaspis* is an uncomplicated genus of four well-characterised species. Furthermore, the genus has been the subject of extensive toxicological research and so a substantial amount of toxin sequence data is now available. In addition, we present LC-MS data for the genus, and test their ability to contribute to the phylogeny of the genus. First, a gene tree reconstructed from 42 *Dendroaspis* three finger toxin amino acid sequences was subjected to gene tree parsimony analysis, which seeks to identify the organismal tree requiring the fewest assumptions of gene duplication and loss events. The resulting organismal phylogeny was congruent with the mitochondrial phylogeny for the genus, but support for the topology was low. This emphasised the importance of complete and even sampling of toxins across species for this method to be of any value. Thus, in view of the extremely uneven sampling of toxin sequences currently available from public databases, toxin amino acid sequences are of limited use for the analysis of the phylogeny larger, more complicated and less well sampled taxa. LC-MS data were found to be useful for elucidating intraspecific relationships, but there were too few shared components to be useful at the interspecific level.

Biology & Evolution of Toxins - Meeting Room 3 - 1445-1500

ABSTRACT NUMBER 17801 2CR4

THE MOLECULAR EVOLUTION OF COLUBROIDEA SNAKE VENOMS

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The evolution of the venomous function of snakes and the diversification of the toxins has been of tremendous research interest and considerable debate. It has become recently evident that the evolution of the toxins in the advanced snakes (Colubroidea) predated the evolution of the advanced, front-fanged delivery mechanisms. Historically, the venoms of snakes lacking front-fanged venom delivery systems (conventionally grouped into the paraphyletic family Colubridae) have been largely neglected. We isolated and characterised a novel three-finger toxin, which we have called 'alpha-colubritoxin', from the Asian Ratsnake *Coelognathus radiatus* (formerly known as *Elaphe radiata*), an archetypal non-venomous snake as sold in pet stores. This potent postsynaptic neurotoxin displayed readily reversible, competitive antagonism at the nicotinic acetylcholine receptor. The toxin is homologous with, and phylogenetically rooted within, the three-finger toxins, previously thought unique to elapids, suggesting that this toxin family was recruited into the chemical arsenal of advanced snakes early in their evolutionary history. We used LC/MS (liquid chromatography, mass spectrometry) to analyze a large number of venoms from a wide array of species representing the major advanced snake clades Atractaspididae, Colubrinae, Elapidae, Homalopsinae, Natricinae, Psammophiinae, Pseudoxyrhophiinae, Xenodontinae, and Viperidae. The results revealed the widespread presence of components of the same molecular weight class, suggesting the ubiquity of three-finger toxins across advanced snakes, with the exclusion of Viperidae. N-terminal sequencing of toxins isolated from other 'colubrid' lineages confirmed the wide spread dispersal. We subsequently used rigorous phylogenetic analysis to examine other toxin lineages to determine recruitment events. The hitherto unsuspected diversity of toxins in all lineages has implications ranging from clinical management of envenomings to venom evolution to the use of isolated toxins as leads for drug design and development. These results support the role of venom as a key evolutionary innovation in the early diversification of advanced snakes and provides evidence that forces a fundamental rethink of the very concept of non-venomous snake. In view of the homology of the toxin-secreting glands of all advanced snakes and their toxins, the fact that Duvernoy's glands represent a primitive condition, and that the derived glands of the front-fanged snakes are independently derived from these, we suggest that the distinction between Duvernoy's glands and venom glands is an artificial one that impedes our understanding of the evolution of the venom apparatus of snakes. For this reason, we propose that the term "Duvernoy's gland " should be abandoned, and that the term "venom gland" be used for the toxin-secreting oral glands of all snakes, regardless of the degree of anatomical specialization in the venom delivery apparatus.

Biology & Evolution of Toxins - Meeting Room 3 - 1500-1515

ABSTRACT NUMBER 11401 2CR5

CLONING AND EXPRESSION OF THE CDNA ENCODING A NOVEL THREE-FINGER TOXIN IN BOIGA DENDROPHILA (MANGROVE SNAKE) VENOM

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Snakes venoms are complex mixtures of pharmacologically active proteins and peptides. At present, venom composition of the opisthoglyphous (rear-fanged) colubrid snakes is largely unknown. Recently, we purified a polypeptide from the colubrid snake (*Boiga dendrophila*) using two-step purification process including cation exchange and reverse-phase HPLC. More than 90% of the sequence was determined by Edman degradation. Based on the available amino acid sequence, degenerated primers were designed to fish out full-length cDNA using RACE (Rapid Amplification of cDNA Ends) technique. The protein, named Boigatoxin, belongs to the structural family of three-finger toxins. It possesses 77 amino acid residues and five disulfide bridges. Of these, four disulfides are conserved among the three-finger toxins and the fifth disulfide is located in the first loop. Thus boigatoxin is similar to non-conventional toxins. However, it structurally differs from all other three-finger toxins in having seven additional residues at its N-terminus which is blocked by a pyroglutamic acid residue. As we are interested in further characterization of the protein, boigatoxin DNA was cloned into pET32a(+) vector and overexpressed in *E.coli*.

Biology & Evolution of Toxins - Meeting Room 3 - 1515-1530

ABSTRACT NUMBER 00502 2CR6

TRIMERUSURUS STEINEGERI VENOM PLASMINOGEN ACTIVATOR STRUCTURE, FUNCTION AND MODEL FOR THROMBOLYTIC AGENTS

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The high selectivity of snake venom proteins for individual blood coagulation factors makes them potentially useful tools to study the mechanisms of action and the structure-function relationships of human coagulation factors. The *Trimeresurus stejnegeri* venom plasminogen activator (TSV-PA) selectively activates plasminogen into plasmin, like tissue-type plasminogen activator (t-PA), cleaving Arg^{561}-Val^{562} bound. However, at variance with t-PA, TSV-PA is insensitive to plasminogen activator inhibitor 1 (PAI-1), as well as several other physiological serine proteinase inhibitors. TSV-PA has been cloned and sequenced from *Trimeresurus stejnegeri* venom glands and it has been expressed in *E. coli* as a recombinant protein that possesses the molecular, enzymatic and physiological properties of natural TSV-PA, excepted that it is not glycosilated. Sequence analysis indicated that TSV-PA is homologous to the catalytic domain of serine proteinase. The 3D structure of recombinant TSV-PA has been determined by crystallographic studies and important structural similarities were observed between TSV-PA and the protease domain of t-PA. Both plasminogen activators possess an acidic edge (DDE 96-98) at the "north" of their active sites that was demonstrated, by directed mutagenesis on TSV-PA, to play a fundamental role in plasminogen recognition. On the other hand and at variance with t-PA, TSV-PA has unexpectedly a Phe residue at the position 193, which restricts the access of its S2' pocket. The replacement of the Phe by a Gly points out the major role of residue 193 in the control of substrate and inhibitor specificity of TSV-PA. Finally, the inability of serpins, in particular PAI-1, to neutralize TSV-PA appears to depend also upon the contribution of a secondary binding site. The substitution of the variable region 1 of TSV-PA for the t-PA one's increases over hundred folds the k_{on} value for the inhibition by PAI-1. Interestingly, the point mutation Phe193Gly associated with the substitution of the variable region 1 of TSV-PA for that of t-PA resulted in a mutant TSV-PA as sensitive than t-PA to PAI-1. These studies on TSV-PA emphasize the interest of snake venom proteins as models and/or tools in haemostasis and thrombosis.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 21101 2PF.2

NO STEREOSPECIFIC CYTOLYTIC EFFECTS OF CUPIENNIN 1A ON DIFFERENT CELL TYPES

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The venom of the spider *Cupiennius salei* contains a diversity of components, which helps to paralyse prey items. Beside a hyaluronidase, neurotoxic peptides and low molecular substances, highly active cationic peptide antimicrobials have been identified and named cupiennins. These linear peptides are composed of 35 amino acid residues and are able to adopt an alpha-helical structure in the presence of membranes and membrane mimicking substances. No stereospecific cytolytic effects of all-D-cupiennin 1a and all-L-cupiennin 1a on Gram + as well on Gram – bacteria, of trypanosomes (bloodstream form) and human erythrocytes are detectable.

Cupiennin 1a is about 17 times less insecticidal than the main neurotoxin CSTX-1 but shows highly synergistic insecticidal effects when administered in a non-toxic concentration together with CSTX-1 in a Drosophila bioassay. The cytolytic peptides in the venom of the spider play a possible dual role: first as protection against microbial infection of the venom glands and second as facilitation of the neurotoxins activity. It is possible that the cupiennins become attracted by the negatively charged basement membranes in insects resulting in an alpha-helical conformation which disturbs membranes and influences cell excitability.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 01302 2PF.2

CHARACTERISATION OF INDIVIDUAL SCORPION VENOM SAMPLES USING SELDI-TOF MASS SPECTROMETRY.

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The proteomic analysis of venoms is increasingly being seen as a useful tool in taxonomic analysis and has been proposed as a complementary method to morphology and behavioural characteristics for species and ultimately sub-species discrimination. However the venom profile and toxicity of individual venomous animal of a given species can vary between different individuals and also from the same specimen following multiple venom extractions over time. It is therefore important to assess this variability between individual specimens with proteomic techniques. In this study, we have chosen to investigate the venom profile of the Indian red scorpion *Mesobuthus tamulus* as a model, using surface enhanced laser desorption / ionization time of flight mass spectrometry (SELDI-TOF MS), which relies on the selective retention of peptides and proteins on a functionalised surface (commercially known as a ProteinChip array). Single specimens of another Indian species (*Heterometrus fulvipes*) and also the African species *Pandinus imperator* were also used, as comparisons.

Red scorpions were collected from Ratnagiri, Chiplun and Aurangabad, in the state of Maharashtra. They were electrically milked and individual venom samples collected. Samples were extracted with de-ionised water, acidified to pH 3.0 with acetic acid, centrifuged at 12,500 x g for 30 min. and the supernatant collected (156-936mg venom protein per scorpion). Supernatants from individual specimens were adsorbed on to either hydrophilic or hydrophobic protein chips (Ciphergen) and analysed by SELDI-TOF MS. The hydrophobic chip provided a useful fingerprint for identifying the origins of individual specimens as from either the Konkan coastal region (Ratnagiri and Chiplun) or from Aurangabad. The hydrophilic chip was further able to identify minor variations between individual specimens. Scorpions from the two regions are also serologically distinct as antivenom (Haffkine) raised against the Aurangabad scorpion does not protect against the Konkan species.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 04102 2PF.2

THE REPORT OF TREATING 54 PATIENTS BITED BY VINCBUCA (TRIATOMA INFESTANS) WITH SHENG NONG SKIN ANTI-VENOM ESSENCE

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In 2002 we studied Vincbuca (*Triatoma infestans*) and Reservorios Del *Trypanosoma cruzi*. All 54 cases treating with Sheng Nong anti—venom essence (Wuzhou snake medicinal liquor) have recovered.There were 14 men and 40 women in these patients. Age of the oldest was 75 years old, and the youngest was 4. In them, 40 cases were wounded on bed, 12 cases at house, a case in storehouse and a case in building site. There were 44 patients only a wound, 4 patients with two wounds, 3 patients with three wounds and 3 patients with four wounds. Common symptoms of this disease were local red, swell, itch and pain. The serious appeared general poisoning symptoms, such as having a fever and aversion to cold, dizzying, feeling bored in chest, vomitting, abdominal pain, and polypnea, etc. After treatment using local hydropathic compress with Sheng Nong skin anti—venom essence, or combining taking Sheng Nong anti—venom crystalline (Wuzhou snake tablet) to patients appeared general poisoning symptoms, four times per day and a gram per time, all cases recovered in three days.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 07304 2PF.2

ISOLATION AND CHARACTERISATION OF AN INSECT-SELECTIVE NEUROTOXIN ω-ATRACOTOXIN-AR1A FROM THE VENOM OF THE FEMALE SYDNEY FUNNEL-WEB SPIDER, *Atrax robustus*

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Recombinant baculoviruses, expressing insect-selective neurotoxins, are currently one of the most promising alternatives for the growing problem of agrochemical-resistance in most agriculturally important pests. Insertion of genes encoding peptide neurotoxins from scorpion, mite and sea anemone sources have led to some increase in potency of these viruses. This project aimed to isolate and characterise a novel insecticidal peptide neurotoxin from the venom of female *Atrax robustus*. Following initial purification with analytical C18 reverse-phase HPLC, fractions were bioassayed for acute insect and vertebrate toxicity in House crickets (*Acheta domesticus*) and isolated chick biventer cervicis nerve-muscle preparations. At high doses, several fractions showed selective insect toxicity by lateroventral injection, and the most potent was selected for further purification, using shallow gradient reverse-phase and anion exchange liquid chromatography. The mass of the purified peptide was determined to be 4003 ± 1 Da using ESI-QTOF mass spectrometry. An LD50 (median lethal dose) of 250 ± 28 pmol/g, and a KD_{50} (median knockdown dose) of 149 ± 16 pmol/g were determined at 48 hours post-injection. Lack of toxicity on chick nerve-muscle preparations was confirmed at concentrations up to 1 mM. Pyridylethylation followed by ESI-QTOF mass spectrometry identified six cysteine residues, with Edman degradation revealing the entire amino acid sequence of 37 residues (Swiss-Prot accession number - P83580). This sequence shows high homology to all known members of the ω-atracotoxin-1 family from Australian funnel-web spiders, particularly to ω-ACTX-Hv1b (100% homology and 89% identity). These toxins selectively block insect voltage-gated Ca^{2+} channels. Importantly, there is complete conservation of the number and spacing of cysteines, as well as of the key residues of the identified pharmacophore of the ω-ACTX-1 family (King et al., 2002). Accordingly, this peptide has been named ω-ACTX-Ar1a, following the nomenclature used for other funnel-web spider toxins. Currently, patch-clamp analysis using dorsal unpaired median neurons isolated from the terminal abdominal ganglia of the cockroach Periplaneta americana is being undertaken to confirm that the insect voltage-gated Ca^{2+} channel is the target site.

King GF, Tedford HW and Maggio F (2002) Journal of Toxicology – Toxin Reviews 21: 359-389.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 07306 2PF.2

CHARACTERISTICS OF THE VENOM OF AUSTRALIAN URODACID AND BUTHID SCORPIONS: VENOM PRODUCTION, COMPOSITION AND TOXICITY

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Australia has some thirty species of scorpions distributed across the families Urodacidae and Buthidae. However very little is known of their venom characteristics. Scorpions from nine species were collected from different habitats across eastern and southern Australia, by a variety of passive and active methods. Of the Buthidae, mainly *Lychas* species were found, chiefly *L. marmoreus*. Of the Urodacidae, only *Urodacus manicatus* and *U. hoplurus* were collected in large numbers. Two new unidentified species of *Urodacus* were also collected from the Simpson Desert in south-west Queensland. Scorpions were maintained in the laboratory in individual containers for 6-24 months, on a varied diet of crickets, cockroaches, flies and woodlice. Venom was extracted periodically by electrical stimulation of the telson (2-25 V, 40 Hz, 1 sec), pooled and freeze-dried. Characteristics such as longevity of individuals in captivity, individual venom yield, dry weight and protein concentration of venom, were recorded and will be presented. Reverse-phase HPLC was used to generate chromatographic profiles of the proteinaceous venom components of the five most abundant species. Using an aqueous/acetonitrile buffer system, acidified with 0.1% trifluoroacetic acid, a continuous gradient from 5-50% acetonitrile was employed to elute components of the venom from an analytical C18 column. Analysis of the resultant chromatogram revealed a similar picture for Buthid venom (*Lychas marmoreus*) as seen with the venom of overseas Buthids, with 10-20 separate peaks observed. In contrast, the venom profiles of the three Urodacids examined (*U. manicatus*, *U. hoplurus* and *U.* species 1) showed incredible complexity, with at least 100-200 poorly-resolved peaks. Adequate separation of components from these *Urodacus* venoms was only successful with a combination of chromatographic techniques (size exclusion and reverse-phase HPLC). The composition of *U. manicatus* and *U. hoplurus* venom from different batches was also compared to determine whether there were any changes related to factors such as sex, season, collection location, and period in captivity. No significant effects were noted. Acute toxicity of the venom was bioassayed in insects by injection and vertebrate toxicity by subcutaneous injection into mice or application to isolated chick biventer cervicis nerve-muscle preparations. Both Urodacid venoms were found to contain lethal vertebrate- and insect-selective neurotoxins justifying further studies to isolate and characterise novel neurotoxins from these Australian scorpion venoms.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 07801 2PF.2

RESEARCH OF TREATING SNAKEBITE BY SHENNONG ANTIDOTE

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1.Sex: male 96 cases, female 40 cases. 2. Age: 0-9: 16 cases, 10-19: 19 cases, 20-29: 29 cases, 30-39: 29 cases; 40-49: 16 cases, 50-59: 13 cases, over 70: 3 cases. 3. Wound position: hand :16 cases, arm: 6 cases, foot: 67 cases, leg: 2 cases, other position: 5 cases. 4.The period before treated: 0-4 hours: 46 cases , 5-8 hours: 18 cases, 9-12 hours: 8 cases, 13-24hours: 18 cases, over 25 hours : 46 cases. 5. Wounded season: spring: 4 cases, summer: 51 cases, Autumn: 55 cases, winter: 26 cases. 6. Teeth traces: 1 trace: 17 cases, 2 trace: 61 cases, 3 trace: 4 cases, two couple of trace: 3 cases, three couple of trace: 1cases, traces blurred: 49 cases, no trace: 1 cases. 7. Species of snakes; *Naja naja* 46 casses. *Bungarus fasciatus* 1 cases, *Bungarus multicinctus* 11 cases, *Agkistrodon halys* 2 cases, *Trimeresurus mucrosquamatus* 27 cases, *Trimeresurus stejnegeri* 36 cases, *Agkistrodon acutus* 2 cases, *Ophiophagus hannah* 7 cases, other kind of snake 4 cases. 8. Clinical classificatin; according to the standards on The Fourth China Congress on Snakebite. mild: 88 cases, serious: 44 cases, critical: 4 cases. 9. Result: all 136 cases recovered.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 07901 2PF.2

THE NURSING OF PATIENTS BITTEN BY COBRA

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The toxin causing muscle necrosis that contains in the cobra poison can make the muscle periosteum necrotic.It is clinically singnificant that during the earlier period the swelled place is cut open for decompression.It is very important in nursing that the wound covered with herb medicine externally and washed can quickly cancel swelling and pain,avoiding have the wounded limbs bound,closely observing the ill symptom with meticulous nursing and adopting corresponding immediate measure can no only save patient`s life,also can preserve patient`s limbs,and minimize the degree of patient`s disease and injury to get the patient recovered as soon as possible.

Key words:wound of cobra;nursing;measures.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 09701 2PF.2

ATOXIGENICITY IN ASPERGILLUS FLAVUS INDUCED BY PHENOLIC NATURAL PRODUCTS.

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All tree nuts are subject to contamination by aflatoxins but walnuts are exceptionally resistant to aflatoxigenesis. The resistance factor(s) are restricted to the seed coat (pellicle) and are not present in the kernel. Chemical analysis of the seed coat has established that the inhibitory activity resides in a complex of hydrolyzable tannins common to all walnut cultivars. In vitro experiments showed that tannin from the cultivar 'Tulare' completely suppressed growth of *A. flavus* at a concentration of 0.5% in the media, with no aflatoxin formed. Aflatoxin biosynthesis appears to be inhibited by gallic acid, produced from the tannin by the action of a tannase known to be present in *A. flavus*. Pure gallic acid reduced aflatoxin levels to ca. 12% of control at a concentration of 200 ppm in media consisting of either walnut or pistachio kernel without seed coat. Treatment of walnut seed coat tissue with anhydrous methanolic HCl yields methyl gallate and ellagic acid, the levels of which can be measured by reverse phase HPLC. Gallic acid levels in seed coat of 'Tulare' and the variety 'Chico', which is susceptible to aflatoxin formation, were determined on a biweekly basis throughout the growing season. Levels in 'Tulare' were significantly higher, and were maintained throughout the growing season, whereas those in 'Chico' declined steadily as the nuts matured. At maturity, 'Tulare' had a gallic acid content of 3.3% (dry weight basis) while the level in 'Chico' was only 1.4%. 'Kerman' pistachio seed coat had 0.5% gallic acid, but 'Nonpareil', 'Mission', and six other almond varieties, only had trace levels (<0.1%). Within the group of tree nut seed coats tested so far, gallic acid content correlated inversely with ability to produce aflatoxin. The hydrolyzable tannins in pistachio are structurally similar to those in walnut, but generate only gallic acid on hydrolysis. Moreover, the tannin is concentrated mainly in the hull, with low levels in specific parts of the seed coat. However, pistachio hull tannin is a potent inhibitor in vitro of *A. flavus* growth, with none being observed at 40 ppm tannin in pistachio kernel/agar media. The evidence indicates that hydrolyzable tannins are capable of inhibiting growth of *A. flavus* and that atoxigenicity is phytochemically induced by biosynthesis and maintenance of high levels of tannins throughout the growing season. Gallic acid, produced in situ by a fungal tannase, is a specific tannin component responsible for suppression of aflatoxin biosynthesis by the fungus and should be amenable to enhancement by conventional breeding or genetic manipulation.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 10102 2PF.2

ADAPTIVE EVOLUTION OF SCORPION SODIUM CHANNEL TOXINS

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Gene duplication followed by positive Darwinian selection is an important evolutionary event at the molecular level, by which a gene can gain new functions. Such an event might have occurred in the evolution of scorpion sodium channel toxin genes (alpha- and beta-groups). To test this hypothesis, a robust statistical method from Yang and co-workers based on the estimation of the nonsynonymous to synonymous rate ratio (w = dN/dS) was performed (Yang et al. 2000; Yang and Nielsen 2000). The results provide clear statistical evidence for adaptive molecular evolution of scorpion alpha- and beta-toxin genes. A good match between the positively selected sites (evolutionary epitopes) and the putative bioactive surface (functional epitopes) indicates that these sites are most likely involved in functional recognition of sodium channels. Our results also shed light on the importance of the B-loop in the functional diversification of scorpion alpha- and beta-toxins.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 10103 2PF.2

PHARMACOLOGICAL PROPERTIES OF THE SCORPION ALPHA-LIKE TOXIN BMK M1 IN TWO DIFFERENT INSECT MODELS

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Previous pharmacological studies using alpha-toxins have already shown their possibilities for developing potent and efficacious insecticides. In this study, the effect of the scorpion alpha-like toxin BmK M1 was investigated on isolated DUM neurons from *Locusta migratoria* and compared with the effect on para/tipE voltage-gated Na+ channels, cloned from *Drosophila melanogaster.* To be able to offer a more thorough insight in the molecular epitopes of channel:toxin interaction, glycine mutants of 5 highly conserved aromatic amino acid residues of recombinant BmK M1 expressed in S. cerevisae S-78 cells were used (Y14G, Y21G, W38G, Y35G and Y42G). Large differences in potency and efficacy were noticed when the two insect models were electrophysiologically compared. The EC50 values on DUM neurons were at least 5 times higher than on para/tipE. Furthermore, obvious differences in the EC50 values within one insect model were observed with the mutant toxins, indicating the importance of aromatic residues for the interaction of the toxin with the voltage-gated sodium channel in *Locusta migratoria* and *Drosophila melanogaster*.

As supported by our data, insects display different pharmacological properties regarding alpha-like toxins when compared to vertebrates. This study, where two different insect models are electrophysiologically compared, reveals the insect-selectivity of BmK M1 and lifts a corner of the veil regarding its ability to discriminate between different species.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 10201 2PF.2

GENE STRUCTURE, ALTERNATIVE POLYADENYLATION AND FOLD RECOGNITION OF THE SCORPINE FAMILY OF ANTIMICROBIAL PEPTIDES FROM OPISTOPHTHALMUS CARINATUS (SCORPIONIDAE)

SY Zhu, J Tytgat

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Scorpine is a unique scorpion venom polypeptide with antibacterial and anti-malaria activities. From the venom gland of the African scorpion, *Opistophthalmus carinatus*, we isolated and identified several cDNAs encoding four new isoforms of scorpine (named opiscorpines). Importantly, we show the existence of multiple mRNAs with variable 3' UTRs for this family of peptides, which may be generated by alternative usage of polyadenylation signals. Sequence analysis and fold recognition allow us to group the scorpine family and scorpion long-chain K-toxins together into a class of polypeptide molecules sharing a similar fold with two distinct domains. The genomic DNA of opiscorpine 3 was also cloned and sequenced. Two large introns were found within the 5' UTR and N-terminal domain of the mature peptide, which is distinct as compared with scorpion neurotoxins. The promoter region was also identified with consensus acting sequence elements at several conserved positions. By combining the gene structure with 3-D fold data, we suggest a role of the scorpine family in the evolution of cecropins and insect defensins.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 10202 2PF.2

EVOLUTIONARY EPITOPES OF HSP90 AND P23 IN SCORPION VENOM: IMPLICATIONS FOR THEIR INTERACTIONS

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The N-terminal domain (residues 1-220) of the molecular chaperone hsp90 is important for ATP binding and interaction with co-chaperone p23. It is clear that p23 binds to hsp90 in an ATP-dependent manner. A six step model has been proposed to explain the interactions among these three molecules. This model predicts a specific conformational change in hsp90 induced by the binding of ATP initiating the binding of p23 to hsp90. However, binding sites between these remain unknown due the lack of mutagenesis data. Here, we try to address this question by combining an evolutionary trace (ET) analysis with molecular docking techniques. ET analysis of 120 hsp90 sequences from diverse species allows us to extract 60 evolutionarily important positions that are obviously clustered in three regions when locating these sites on the three-dimensional structure. From 25 p23 and related sequences, ET identifies 14 key residues which form two separate clusters. Significantly, among them only two ET positions are exposed to the molecular surface. Using molecular docking, one interesting interface is characterised which includes part of the ET residues of both hsp90 and p23. On the basis of these observations and in reference to the six-step model, we propose a new binding model which fully integrates the experimental data currently available.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 10203 2PF.2

EVOLUTIONARY TRACE ANALYSIS OF SCORPION TOXINS SPECIFIC FOR K-CHANNELS

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Scorpion á-K+ channel toxins are a large family of polypeptides with a similar structure but diverse pharmacological activities. Despite many structural and functional data available at present, little progress has been made in understanding the toxin's molecular basis responsible for the functional diversification. To identify sites probably involved in the functional diversity of á-KTx family, we analysed this family of sequences using the evolutionary trace method. This analysis highlights one channel-binding surface common for all the members. This surface is composed of one conserved lysine residue at position 29 assisted by other residues at positions 10, 26, 27, 32, 34 and 36. Of them, positions 29, 32 and 34 have been reported to be the most major determinants of channel specificity. Interestingly, another contrary surface was also observed at a higher evolutionary time cut-off value, which may be involved in the binding of ERG channel-specific toxins. The good match between the trace residues and the functional epitopes of the toxins suggest that the ET results reported here can be applied to predict channel-binding sites of the toxins. Moreover, because the side-chain variation in the trace positions is strongly linked with the functional alteration and channel-binding surface transfer of á-KTx family, we conclude that our findings should also be important for the rational design of new toxins targeting a given potassium channel with high selectivity.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 11002 2PF.2

A SUBFAMILY OF ACIDIC Á-K+ TOXINS

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Three homologous acidic peptides have been isolated from the venom of three different *Parabuthus* scorpion species, *P. transvaalicus, P. villosus* and *P. granulatus*. Analysis of the primary sequences reveals that they structurally belong to subfamily 11 of short-chain á-K+ blocking peptides. These toxins are 36 to 37 amino acids in length and have 6 aligned cysteine residues, but they differ substantially from the other á-KTx's because of the absence of the critical Lys27 and their total overall negative charge. PBTx1, which has been expressed by recombinant methods, has been submitted to functional characterization. In spite of the lack of the Lys27, this toxin blocks several Kv1-type channels heterologously expressed in Xenopus oocytes, but with low affinities (micromolar range). Since a relationship between the biological activity and the acidic residue substitutions may exist, we set out to elucidate the relative impact of the acidic character of the toxin and the lack of the critical Lys27 on the weak activity of PBTx1 towards Kv1-channels. To achieve this, a specific mutant, named rPBTx1 T24F/V26K, was made recombinantly and fully characterized on Kv1-type channels heterologously expressed in Xenopus oocytes. rPBTx1 T24F/V26K displays an affinity towards Kv1.2 and Kv1.3 channels in the nanomolar range.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 13401 2PF.2

DETECTION OF GELATINOLYTIC, CASEINOLYTIC AND HYALURONIDASE ACTIVITIES IN SOME NEUROTOXIC SNAKE VENOM

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Snake neurotoxic venoms are a complex mixture of toxins which can cause blockade of neuronal conduction, neuromuscular junction transmission or the contractile response of muscle. Some of these toxins can also disturb other parts of the nervous system, function of smooth muscle and interfere with coagulation. The presence of prothrombin-activing enzymes and lipases (most of these are phospholipases A2) have also been described. We have examined and compared venom enzymatic activity on gelatin, casein and hyaluronic acid substrates from eight species of snake from Australia and Africa (*Bitis arietans, Naja melanoleuca, Naja mossambica, Bitis nasicornis, Notechis scutatus, Pseudechis australis, Pseudechis guttatus* and *Pseudonaja textilis*) which have neurotoxic activity. By SDS-PAGE (12.5%), using 15 mg of venom, many components appear distributed across the entire molecular weight range (7- 209 kDa). However the majority of venom components are located between 30-209 kDa (silver stain). Venom enzymatic activities were assayed using SDS-PAGE in presence of both gelatin and casein (2 mg/ml) and hyaluronic acid (170 μg/ml) as substrate. The *P. textilis* has components that degrades gelatin (around 50 kDa) and casein (approximately 70 kDa). The *B. nasicornis* venom also has gelatinolytic activity (components with range of 35-70 kDa), however they did not show caseinolytic activity. Venoms of *N. scutatus* and *P. guttatus* only present caseinolytic activity around 50-80 kDa and 50 kDa, respectively. The hyaluronidase activity was only detected in *B. arietans* (around 84 kDa), *B. nasicornis* (approximately 84 kDa) and *N. melanoleuca* (around 80 kDa). *N. mossambica* and *P. australis* did not show any of these enzymatic activities. We used *Agkistrodon bilineatus* snake venom from Mexico and *Loxosceles gaucho* spider venom from Brazil (only to assay hyaluronidase activity), which cause extensive local damage as control. The A. bilineatus venom showed components with strong caseinolytic (between 35-50 kDa) and gelatinolytic activities (around 33, 35-50 and 69 kDa), no hyaluronidase activity was detected. The *L. gaucho* venom showed a intense hyaluronidase activity around 44 kDa. In conclusion, elapid and viperid venom enzymes exhibited a degree of substrate specificity. The enzymatic activity found in the venoms we examined, may contribute in some cases, to local damage and to the movement of venom components in connective tissue.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 15403 2PF.2

PHARMACOLOGICAL CHARACTERIZATION AND LOCALIZATION OF THE BMTX3 AND AMMTX3 TARGET IN RAT BRAIN

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The scorpion toxin (BmTX3), isolated from the *Buthus martensi* Karsch venom, constitutes with two other toxins (Aa1 and AmmTX3 from the scorpion *Androctonus australis* and *Androctonus mauretanicus mauretanicus*), the new family alpha-KTx15 of scorpion short-chain toxins active on K^+ channel. A specific binding was demonstrated on rat brain synaptosomes with 125I-synthetic BmTX3 and native 125I-AmmTX3. A maximum of 24 fmol/mg of protein, with a dissociation constant (KD) of 0.2 nM and 0.06 nM respectively were obtained under equilibrum binding conditions. These KD were confirmed by kinetic and competition experiments. These toxins and Aa1 bind to the same site in rat brain. However, a panel of toxins (described as specific ligands of different K^+, Na^+ and Ca^{++} channels) were assayed in competition experiments with 125I-BmTX3 and proved unable to displace the toxin from its binding site. Thus, it could be claimed that these toxins were new ligands for a not yet identified target in rat brain. Ontogenesis of 125I-BmTX3 binding sites in rat brain was achieved. In adult brain, high density of BmTX3 receptors was found in the striatum, hippocampus, superior colliculus, and cerebellum. Striatum neurons in culture were used to perform further experiments of electrophysiology and to identify the brain target of this new class of toxins. When 1 μM BmTX3 (KD=54 nM) or AmmTX3 (KD=134 nM) were applied on striatum neurons in culture, an A-type K^+ current disappeared, whereas the sustained K^+ current remained unaffected. RT-PCR was performed on voltage activated K^+ channels of the striatum neurons: Kv 1.1, Kv1.2, Kv1.3, Kv1.4, Kv1.5, Kv3.4, Kv4.1, Kv4.2 and Kv4. alpha-subunits are present. In literature, A-type currents are defined by the association of: (1) Kv1.4, Kv3.4, Kv4.1, Kv4.2 and Kv4.3 alpha-subunits ; (2) Kv 1.1, Kv1.2, Kv1.3, Kv1.5 alpha-subunits with an auxiliary cytoplasmic bêta-subunit. To further characterize the target of BmTX3, some toxins known to block Kv1.4, Kv3.4 and Kv4. family have been applied with BmTX3 in whole-cell patch clamp experiments. Even in the presence of a cocktail of these toxins, part of the A-type K^+ current remained and was blocked only by BmTX3. Also, cDNA of these cloned channels were injected in mammalian cells (HEK and COS cells) and the currents expressed studied in electrophysiology experiments. In parallel, some biochemical experiments have been performed with BmTX3 cross-linked to its target on rat brain synaptosomes.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 15502 2PF.2

A POSITION-SPECIFIC DELETION AS AN EVOLUTIONARY LINK BETWEEN LONG- AND SHORT-CHAIN SCORPION TOXINS

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Most scorpion toxins can be classified as either long-chain toxins (60 to 70 amino acid residues), which target voltage-gated Na^+ channels, or short-chain toxins (less than 40 amino acid residues), which target K^+ channels.

Initial studies on the genetics of scorpion toxins led to the suggestion of a one gene - one toxin organisational model, with the idea of a putative common ancestor leading to an hypervariable gene family. A unique intron, located close to the end of the signal peptide, seems to be a conserved feature. Furthermore, the amino acid sequences are more highly conserved in the signal peptides than in the toxins themselves. We took advantage of this conservation and found by subtractive selection new clones (AJ308442, AJ308443, and AJ308444) that, we believe, define an evolutionary link between long- and short-chain toxins. They encode shorter peptides (53 amino acid residues). Closer comparison with clones encoding long-chain toxins (AJ308440 and AJ308441) revealed a unique "CC" deletion at the proline codon of the LPD sequence that resulted in a frame shift. This frame shift resulted in a premature stop codon, such that protein translation ended by LR. The presence of the arginine residue is canonical concerning scorpion toxin precursors. It is known that position-specific codon conservation exists in hypervariable gene families, as recently documented in conotoxins [1]. We have aligned the amino acid sequences of 48 long-chain scorpion toxins from the SwissProt database and visualised at the best the consensus sequence (77 positions) using LOGO. High variability is observed in the inter-cysteine loops, which indicates that adaptive evolution has taken place as for the conotoxins, in which diversification operates at an extraordinary high rate [2]. However, there were position-specific conserved codons within the loops and, in peculiar, the leucine codon of the LPD sequence. All scorpion toxins share a common structural motif named CSH for Cysteine Stabilised α Helix. This peculiar leucine residue is at a structural « hinge » position. In an evolutionary context, we think that it is an interesting observation and believe that this is an evolutionary link between long- and short-chain scorpion toxins.

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Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 15801 2PF.2

DETECTION OF CALLOSELASMA RHODOSTOMA AND NAJA KAOUTHIA SNAKE VENOM BY DOT-ELISA

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The treatment of snake-bite using species-specific antivenoms requires identification of the snake venom. One method to achieve this is to use ELISA technique. However, ELISA on microtiter plate has some disadvantages including long incubation time and the requirement of expensive instruments. The objective of this study is to develop a practical method for detection of snake venom by dot- ELISA on nitrocellulose paper using *Calloselasma rhodostoma* and *Naja kaouthia* venoms as models. Immunoglobulin (IgG) against *C. rhodostoma* and *N. kaouthia* venoms were prepared from hyperimmunized horse by affinity purification. These antibodies were then immobilized on nitrocellulose paper. Several parameters including concentration of IgG for immobilization on nitrocellulose paper , time for incubation and washing , volume and concentration of reagents and substrate for enzymatic detection were optimized. The sensitivity of the detection was 5 μg/ml for *C. rhodostoma* and 1 μg/ml for *N. kaouthia*. No cross- reaction between the two snake venoms was observed between 0.2- 25 μg/ml. For comparison , the same ELISA were performed in microtiter plates and showed detection limit of 1 ng/ml for *C. rhodostoma* and *N. kaouthia* venoms. In spite of poorer sensitivity, the developed dot-ELISA technique is less time consuming and requires no special instrument as well as using small amount of sample (1-5 μl). This might be advantageous for field-test. Further studies to improve sensitivity and using for snake species identification kit are in progress.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 16301 2PF.2

ANTIBACTERIAL COMPONENTS IN THE HEMOLYMPH OF INDIAN SCORPIONS

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Hemolymph of both *Buthus tamulus* and *Heterometrus fulvipes* were evaluated for their anti-bacterial properties. The acidified hemolymphs from the Indian scorpions were fractionated on Oasis HLB C18 SepPak column followed by RP-HPLC purification of the active fractions. The anti-bacterial was tested against the Gram-ve, *Escherichia coli* and the Gram+ve, *Micrococcus luteus* bacterial stains. Several purified hemolymph fractions showed inhibitory effect on the bacterial growth. Among them, only one fraction was found to inhibit the growth of both *E. coli* and *M. luteus*. This polypeptide is being further characterized by N-terminal sequencing and cDNA cloning.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 17101 2PF.2

BIOACTIVE MOLECULES FROM THE SALIVA OF THE AUSTRALIAN PARALYSIS TICK

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The Australian Paralysis Tick, *Ixodes holocyclus*, is responsible for thousands of cases of tick paralysis in domestic animals each year (including a fatality rate of 5% in treated dogs) and causes a range of diseases in humans. The tick produces a saliva with anti-haemostatic, anti-inflammatory and immunomodulatory properties which enables it to feed off the host for several days. The only component successfully isolated previously was a neurotoxin. From information available for other Ixodidae (hard ticks) a process for the analysis and purification of crude saliva and salivary gland extracts was devised. Of particular interest were salivary components with neurotoxic or cardiotoxic properties and molecules with characteristics similar to conotoxins and arachnid neurotoxins (molecular weight 5-10kDa, multiple, disulfide bond-linked peptides), lipocalins (molecular weight 15-20kDa, amine-binding) and kininases (enzyme activity). It is hypothesised that one or more of these salivary components is not effectively neutralised by the commercial tick antiserum and might contribute to the lethality of tick paralysis.

Preliminary results from gel electrophoresis and bioassays show that: the range of peptides/proteins produced by this species is greater than previously thought; that there is significant variation between ticks from different regions/hosts; that TAS binds to only large molecular weight components; that all secreted salivary components and biological activity can be regained from frozen whole tick salivary glands; and that conotoxin-like peptides, lipocalins and kininases are produced in *I. holocyclus* saliva.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 17301 2PF.2

CLINICAL FEATURES OF SEVERE ENVENOMING BY THE MALAYAN KRAIT (BUNGARUS CANDIDUS) IN SOUTH VIETNAM

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The Malayan krait (*Bungarus candidus*) is one of the most dangerous land-snake in the world with high mortality rate chiefly due to its severe neurotoxic envenomation. The patients often recover very slowly. Antivenom for this snake is not available so far in Vietnam. Here, we report clinical features of seven cases of severe envenomation by *B.candidus* admitted to Cho Ray Hospital, Vietnam. Most patients were bitten during their sleep. The local symtomps were minimal. The venom detection kit for four common snakes of Vietnam (*Naja naja, Ophiophagus hannah, Trimeresurus popeorum* and *Calloselasma rhodostoma*) gave no positive results. Within two hours after the bites, patients developed systemic symptoms viz. progressive ptosis, dysphagia, dysphonia, sore throat, hypersalivation and breathlessness. Later, apnoea and paralysis occurred which required artificial ventilation. Pupils were fully dilated and did not react to light. Two patients developed symptoms of hypertension which required antihypertensive drugs. The slight increase in hypertension on the third day in other two patients was recorded initially which was followed by severe hyponaetremia, later. Serum osmolality was low, whereas increase of sodium elimination in urine was confirmed. Etiology of hyponaetremia might be due to the syndrome of inappropriate antidiuretic hormone secretion (SIADH). However, the actual mechanism was not established yet. Secondary pneumonia was a common complication during the period of artificial ventilation. The mortality rate was 28.6% (2/7). In conclusion, neurotoxic envenomation caused by *B. candidus* was normally serious and prolonged. Hypertension and hyponaetremia were observed and treated effectively. Antivenom treatment for this snake should be made available and used soon after envenomation to prevent prolong the period of ventilation.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 18601 2PF.2

DEVELOPMENT OF A FLUORESCENT ASSAY FOR CLOSTRIDIAL NEUROTOXINS PROTEOLYTIC ACTIVITY: TETANUS AND BOTULINUM SEROTYPES A AND E

Perpetuo E A[1] , Juliano L,[2] Fratelli F[3], Prado SMA[3] , Lebrun, I [1]

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Clostridial neurotoxins (tetanus-TTx and botulinum-BoNT) are zinc metalloproteases that cleave and inactivate cellular proteins essential for neurotransmitter release. This occours by cleavage of toxin light chain on conserved proteins involved in exocytosis of neuron vesicles, such as synaptobrevin. In order to develop a sensitive assay to quantify the proteolytic activity of TTx and BoNT A and E, we designed fluorescent substrates based on target proteins sequences hydrolysed by them. The substrates are the fragments of rat synaptobrevin, sintaxin IA and SNAP-25, which were modified by introduction of the fluorescent o-aminobenzoyl (Abz) in N-terminal and ethylenediamine dinitro-phenyl (EDDnp) in C-terminal positions. The cleavage of a single peptidic bond by toxin active chain is directly quantified by measuring the strong fluorescence of the formed N-terminus peptide fragment metabolite. This is a rapid and quick assay to measure the proteolytic activity of TTx and BoNT A and E. Moreover, amounts of ng of neurotoxin could be detected by this method. Solutions of holotoxin of TTx, BoNT A and E (10 ng) obtained from Section of Anaerobic Vaccines from Butantan Institute, were analysed by SDS-PAGE to verify their purity and after incubated with the fluorescent substrates (1mM) in reaction buffer (50 mM Tris-HCl pH 7,5) at 37°C for 10 minutes. A hydrolysis curve was plotted and the fragments were collected and submitted to aminoacid analysis and mass spectrometry to verify the cleavage point. The cleavage of SNAP-25 by the toxin active chain occoured selectively between residues Gln 197-Arg 198 by BoNT A and Arg 180-Ile 181 by BoNT E, and the cleavage of synaptobrevin by TTx occoured between Gln 76-Phe 77 residues, as described in the literature (Biotechnol Appl Biochem. 36: 155-61, 2002). Some structural modifications were carried out in substrate based on synaptobrevin, to check the specificity of TTx. We verified that the TTx hydrolyses preferencially the peptidic bond Gln 76-Phe 77, but in absence of this bond, TTx can hydrolyse other peptidc bonds. The kinetic constants (Km) varied between 2,4 to 5,7 μM. The fluorescent assays were selective and gave low background readings. Besides, this method did not employ antibodies or reverse-phase extraction steps, so could be easily adapted to a production process or other quantitative diagnostic assays. Supported by FAPESP, CNPq and Butantan Foundation.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 21902 2PF.2

CLONING SEQUENCING AND EXPRESSION OF BOTHROPSTOXIN-1, A K49 MYOTOXIN

Spencer, P.J. [1]; Webb, R.P. [2]; Lomonte, B. [3]; Angulo, Y. [3]; Campos, L.A. [1]; Moura da Silva, A. M. [4] & Smith, L.A. [2].

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With the aim of obtaining recombinant Bothropstoxin-1 for functional studies, we constructed a cDNA library wich was then screened with a specific probe. A positive clone was then circularized and sequenced, yielding previously unreported 5'and 3' untranslated regions, a 48 bp domain encoding for signal peptide and the mature protein encoding region. The deduced aminoacid sequence was in complete agreement with the previously obtained by Edman degradation. The open reading frame for the toxin was subcloned in the pET 24 expression vector and transformed in BL21 *E. coli*. After induction, the recombinant product was obtained as inclusion bodies, refolded and purified by ion exchange chromatography. Nor electrophoresis analysis neither reverse-phase chromatography revealed any differences between the native and recombinant toxins. When assayed for activity in vivo and in vitro, no differences were observed between the native toxin and its recombinant counterpart. This might represent a first step towards obtaining properly refolded mutants for structure-function studies.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 22501 2PF.2

THE EFFECTS OF ELAPID SNAKE VENOMS ON THE CELLULAR BIOLOGY OF TUMOUR-ASSOCIATED MICROVASCULAR ENDOTHELIAL CELLS (TAMECS) IN VITRO

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Snake venoms are veritable cocktails of constituents which act in concert to bring about prey immobilisation and digestion. One of the major targets of these pharmacologically active agents is the haemostatic system; venoms disrupt haemostasis by either potentiating or attenuating coagulation or by damaging the walls of blood vessels to cause haemorrhage. It has been shown that several classes of venom components specifically target the endothelial cells lining blood vessels. Adhesion to the extracellular matrix via specific moieties is known to be fundamental for endothelial cell proliferation, motility and viability especially during nascent vessel formation. It has been shown that interference with these adhesive mechanisms not only prevents vessel formation but can lead to apoptosis of vascular endothelial cells and regression of newly-formed blood vessels. Several adhesion inhibitors derived from the venoms of Viperidae and Crotalidae species are capable of eliciting an apoptotic response in endothelial cells in vitro and in vivo.

This study aimed to determine the effects of various Elapidae snake venoms on microvascular endothelial cells. Selected venoms were applied in varying dilutions to tumour-associated microvascular endothelial cells (TAMECs) isolated from a rat mammary adenocarcinoma and normal microvascular endothelial cells. Venom-treated cultures were observed over a 6-hour time period via phase microscopy for morphological changes characteristic of adhesion inhibition and apoptotic cell death. The results demonstrated that venoms from selected Elapidae snake species induced marked cellular changes in TAMECs, which included inhibition of cellular adhesion to the substrate and apoptotic cell death, similar to those observed with venoms of Viperidae and Crotalidae species. The exact mechanisms of action are unclear, however, inhibition of adhesion mechanisms may be involved.

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 22901 2PF.2

THE CARDIOVASCULAR EFFECTS OF THE VENOM FROM VIPERA LEPTINA

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The *Vipera leptina* is one of the third most venomous snakes in the Iran plateau; however, little information on the cardiovascular effects of the venom is available. The changes in the mean arterial blood pressure of anaesthetized rats following the administration of *Vipera leptina* venom were recorded. Effects of the venom on perfusion pressure in rat mesenteric bed and on contractions of rat isolated right atrium were also investigated. In anaesthetised rats, 1 mg/kg (i.v.) venom produced rapid respiratory and cardiovascular collapse while 0.5 mg/kg (i.v.) venom produced only a small transient decrease in mean arterial blood pressure. Exposure of the isolated rat mesenteric bed precontracted with phenylephrine to the venom (0.1-3 mg/ml) caused a significant reduction in perfusion pressure. Exposure of rat isolated right atrium to venom (0.1-3 mg/ml) resulted in a transient increase followed by a sustained reduction in the amplitude and frequency of spontaneous contractions. The transient positive inotropic and chronotropic effects were abolished when the preparation was preincubated with propranolol but not with tolazoline. L-NAME pretreatment attenuated the vascular hyporeactivity to phenylephrine induced by the venom in the rat isolated mesenteric vascular bed. This suggests that the consequence release of nitric oxide (NO) caused by the venom may be involved in its hypotensive effect.

Key words: Vipera leptina, Venom, Rat, Cardiovascular function

Poster Session 2PF.2 - Glass Foya - 1100-1140

ABSTRACT NUMBER 16701 2PF.2

A NEW PURIFICATION METHOD OF CLOSTRIDIUM BOTULINUM NEUROTOXIN TYPE B USING IMMUNO-AFFINITY CHROMATOGRAPHY.

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Botulinum neurotoxin, which is produced by *Clostridium botulinum* and classified as seven serotypes (A to G), has been recognized as a potential therapeutic agent based on its clinical studies. Among them, botulinum toxin type B was approved as a therapeutic agent for treatment of neurological disorders by FDA in 2000. Until now, classical procedures involving several precipitation and resuspension steps and a couple of chromatographic steps have been available to purify botulinum toxin B. In this study, we successfully established a new method by using immuno-affinity chromatography with BTBH-N1 mouse monoclonal antibody, which is specific for heavy chain of botulinum neurotoxin type B. As determined by SDS-gel electrophoresis, Western blotting analysis and mouse lethality test, it was shown that feasibly active neurotoxin for clinical use was highly purified. From the results, it is concluded that this new method will be simple, effective and specific as compared to other methods previously reported and might be useful for manufacturing of botulinum toxin type B. [This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea. (02-PJ2-PG4-PT01-0032)]

WEDNESDAY September 17th

SCIENTIFIC PROGRAMME

Plenary Session 3AH - Hall C - 0900-1100

Session Chairperson: Michael Parker

3AH1 Titia K. Sixma
NEUROTOXIN BINDING TO NICOTINIC ACETYLCHOLINE RECEPTORS ANALYSED THROUGH STRUCTURAL STUDIES OF ACHBP

3AH2 Lourival D. Possani
RECENT ADVANCES ON THE KNOWLEDGE OF SCORPION TOXINS

3AH3 Roseanne Skalicky
THE PLATYPUS - A DANGEROUS BREED

Inivited Lecture Session 3BH - Hall C - 1120-1235 - Scorpion Toxins

Session Chairperson: Lourival Possani

3BH1 Dalia Gordon
SODIUM CHANNEL FEATURES THAT CONFER DIFFERENT SENSITIVITIES TO SCORPION TOXINS

3BH2 Michael Gurevitz
THE FUNCTIONAL SURFACE OF SCORPION TOXINS AND ELEMENTS THAT CONFER SELECTIVITY TO INSECT AND MAMMALIAN SODIUM CHANNELS

3BH3 Hervé Rochat
MAUROTOXIN, A MOLECULAR MODEL OF SCORPION TOXIN ENGINEERING

Inivited Lecture Session 3BM - Meeting Room 1&2 - 1120-1235 - Scorpion Toxins

Session Chairperson: Paul Alewood

3BM1 Gopalakrishnakone P
GLOBAL GENE EXPRESSION PROFILING OF HUMAN GENOME FOLLOWING EXPOSURE TO TOXINS - TOXINOGENOMICS

3BM2 David J Craik
DISCOVERY OF CIRCULAR PROTEIN TOXINS FROM BACTERIA, PLANTS, AND ANIMALS.

3BM3 E Hawrot
NMR-BASED STRUCTURAL ANALYSIS OF α-NEUROTOXINS BOUND TO COGNATE PEPTIDES DERIVED FROM NICOTINIC ACETYLCHOLINE RECEPTOR SEQUENCES PROVIDES INSIGHTS INTO THE STRUCTURAL BASIS FOR TOXIN-RECEPTOR RECOGNITION.

Congress Trip

Please promptly board your bus at the conclusion of Sessions 3BH and 3BM for the commencement of the official Congress trip. Details of the trip will be given on the day. All Congress participants and registered accompanying persons are encouraged to participate in this trip. A packed lunch will be provided. We aim to depart before 1300. Our return time will be advised on the day, but may extend into the evening. By all means bring your cameras and if you wish, money.

WEDNESDAY September 17th

Plenary Lecture 3AH.1 - Hall C - 0900-0950

ABSTRACT NUMBER 05101 3AH1

NEUROTOXIN BINDING TO NICOTINIC ACETYLCHOLINE RECEPTORS ANALYSED THROUGH STRUCTURAL STUDIES OF ACHBP

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The first neurotransmitter receptors to be described were the nicotinic acetylcholine receptors. These pentamers of homologous subunits have served as prototypes for ligand-binding ion channels and their complicated ligand-binding and ion-channel properties have been described in exquisite detail over the past 40 years. Several classes of protein toxins, such as the a-neurotoxins and the conotoxins have been critical for this analysis. Detailed atomic coordinates of the receptor, however, are slow in coming. The water-soluble acetylcholine binding protein (AChBP) from the snail *Lymnaea stagnalis* is a homolog of the ligand binding domains of nicotinic receptors with very similar pharmacology. It binds to a-bungarotoxin with high affinity.

Based on the crystal structure of AChBP we can explain the biochemical data on the pentameric ligand-gated ion channels in three-dimensions. The ligand-binding site is found at the interface of two subunits, consisting of a 'cage' of mostly hydrophobic or aromatic residues provided by six different regions in the two subunits. Based on this crystal structure the binding α-bungarotoxin can be modelled to explain many of the recognition features of the toxins.

Plenary Lecture 3AH.2 - Hall C - 0950-1030

ABSTRACT NUMBER 05901 3AH2

RECENT ADVANCES ON THE KNOWLEDGE OF SCORPION TOXINS

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Scorpion venoms contain a great variety of different biological active components. The most widely known are peptides that recognize Na^+- and K^+-channels of excitable cells. Among the newly discovered peptides are the Ergtoxins, peptides capable of blocking K^+-channels from the family of genes ether-a-go-go (ERG). The first described was Ergtoxin-1, a 42 amino acid long peptide (Gurrola et al., FASEB J. 13:953-962, 1999; Scaloni et al., FEBS Lett. 479:156-157, 2000). We have just discovered that the venom of the Mexican scorpions of the genus *Centruroides* contain a large number of ergtoxin-like peptides (Lecchi et al., J. Neurosci. 22:3414-3425, 2002; Pardo-López et al., FEBS Lett. 510:45-49, 2002 and J.Biol.Chem. 277:16403-16411, 2002; Corona et al., FEBS Lett., 532:121-126, 2002). They conform at least 4 independent sub-families of peptides. Interaction of these peptides with HERG-channels will be reviewed. Structural, physiological and evolutionary aspects of these peptides will be also discussed. Additionally, newly discovered peptides in venoms from the Brazilian scorpions of the genus *Tityus* showed that they contain peptidic toxins that recognize Kv1.3 channels, where the presence of the dyad amino acid residues proposed to be essential for activity are missing (Batista et al., BBActa 1601:123-131, 2002). Novel Na^+-channels specific scorpion toxins recently isolated will be also mentioned in respect to their structural and functional activities (Corona et al., BBActa in press).

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Plenary Lecture 3AH.3 - Hall C - 1030-1100

ABSTRACT NUMBER 01501 3AH3

PLATYPUS - A DANGEROUS BREED

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The platypus is a unique Australian mammal that, although often shy, can cause a debilitating envenomation. I will present two clinical cases of platypus envenomation along with a review of all published cases of envenomation and a summary of recent developments regarding the venom's composition and it's mode of action. A treatment plan based on this information is proposed.

Invited Lecture 3BH.1 - Hall C - 1120-1145

ABSTRACT NUMBER 01501 3BH1

SODIUM CHANNEL FEATURES THAT CONFER DIFFERENT SENSITIVITIES TO SCORPION TOXINS

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At least nine different Na channel (NaCh) subtypes are expressed in mammalian excitable cells, but their unique role and developmental pattern are poorly understood. Scorpion alpha-toxins distinguish among NaCh subtypes and are suitable probes for analysis of NaCh distribution. The sensitivity of various neuronal NaChs (Nav1.2, 1.6, 1.7 expressed in *Xenopus* oocytes) varies greatly to three a-toxins: the anti-mammalian Lqh2; the anti-insect, LqhaIT; and the alpha-like, Lqh3. In contrast, the rat skeletal muscle channel (Nav1.4) is similarly sensitive to the three toxins. To clarify the molecular basis of this sensitivity, we focused in Nav1.4 on S3-S4 of domain-4 (D4), known to be involved in receptor site-3, and found that substitution of Asp1428 and Lys1432 affected differently the binding of the three alpha-toxins. Surprisingly, Asp/Glu substitutions dictated the sensitivity of various NaChs to Lqh3 and LqhaIT, but not to Lqh2. Scorpion beta-toxins bind receptor site-4, which is mainly associated with D2. To determine whether D2 dictates the selectivity of beta-toxins to various NaChs, a rat brain NaCh (Nav1.2) chimera bearing D2 of Drosophila Para NaCh was constructed. Whereas Nav1.2 is insensitive to anti-insect excitatory and depressant beta-toxins, the Nav1.2-ParaD2 chimera became sensitive to the excitatory toxin, AahIT, and to a novel beta-toxin, Lqhb1, but was insensitive to depressant toxins. These results suggest that more than a single channel domain forms receptor site-4 and that the various beta-toxins interact differently with this site. Thus, subtle variations in channel receptors that determine differences in sensitivity to scorpion toxins, combined with data on toxin functional surfaces, may be instrumental for the design of channel-targeted drugs.

References: Gilles et al (1999) J Neurosci 19:8730-9; Gilles et al (2000) Eur J Neurosci 12:2823-32; Chen et al (2002) Eur J Neurosci 16:1-5; Shichor et al (2002) J Neurosci 22, 4364-71.

Invited Lecture 3BH.2 - Hall C - 1145-1210

ABSTRACT NUMBER 01701 3BH2

THE FUNCTIONAL SURFACE OF SCORPION TOXINS AND ELEMENTS THAT CONFER SELECTIVITY TO INSECT AND MAMMALIAN SODIUM CHANNELS

Michael Gurevitz, Dalia Gordon, Izhar Karbat, Lior Cohen, Michael Turkov, Roy Kahen, Nitza Ilan

Department of Plant Sciences, George S. Wise Faculty of Life Sciences, Tel-Aviv University, Ramat Aviv 69978, Israel

The functional surface of scorpion toxins representing all major pharmacological groups (anti-mammalian alpha, alpha-anti-insect, alpha-like, anti-mammalian beta, anti-insect depressant and excitatory) was elucidated by mutagenesis followed by toxicity, binding, electrophysiological, and structural assays. Despite the vast differences in their effects on voltage-gated sodium channels, in all toxins the loop preceding the alpha-helix motif and the C-tail are important for function, which may result from their common ancestry and structural resemblance. The variation in selectivity of toxins that belong to the same pharmacological group to different NaCh subtypes is conferred by subtle differences on the functional surface. Accordingly, structural elements that dictate the preference of alpha-toxins to either insect or mammalian brain sodium channels were illuminated and verified by exchange of active sites leading to inversion of selectivity. In beta-toxins, mutagenesis of the anti-insect excitatory toxin, Bj-xtrIT, and the anti-mammalian beta-toxin, Css4, highlighted their functional surface with common motifs that seem to be the 'pharmacophore' of the beta-toxin class. In addition, a conserved negatively charged residue involved putatively in 'trapping' of the channel voltage sensor has been illuminated. These studies contribute to clarification of the mechanism by which scorpion toxins affect NaChs, are crucial for determining the interacting faces between the toxins and their NaCh receptor sites, and raise new possibilities for insecticide and drug design.

Invited Lecture 3BH.3 - Hall C - 1210-1235

ABSTRACT NUMBER 00401 3BH3

MAUROTOXIN, A MOLECULAR MODEL OF SCORPION TOXIN ENGINEERING

Hervé Rochat and Jean-Marc Sabatier

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Maurotoxin (MTX) is a scorpion toxin isolated from the venom of *Scorpio maurus palmatus*. This basic 34-mer peptide is cross-linked according to an uncommon pattern of disulfide bridges (C1-C5, C2-C6, C3-C4, and C7-C8) that replaces the conventional pattern (C1-C4, C2-C5, C3-C6, and C4-C8) of other alpha-KTx 6 toxins. This renders MTX a unique and interesting peptide to address structure-activity relationships, as well as to investigate the process of disulfide bridge formation in vitro and its resulting effects on both peptide structure and bioactivity towards the various potassium channels. Using an innovative strategy of solid-phase peptide synthesis, a MTX variant with a conventional pattern of disulfide bridging was produced that exhibits some marked changes in blocking efficacies towards the target ion channels. Computed docking analyses suggest that the disulfide bridge organization of MTX contributes to its pharmacology by guiding the spatial positioning of certain key residue side-chains.

Invited Lecture 3BM.1 - Hall C - 1120-1145

ABSTRACT NUMBER 06801 3BM1

GLOBAL GENE EXPRESSION PROFILING OF HUMAN GENOME FOLLOWING EXPOSURE TO TOXINS - TOXINOGENOMICS

Gopalakrishnakone P, Srinivasan KN, Zhong S, Pachiappan A, Qi Z and Prasad HSR

Venom and Toxin Research Programme, Faculty of Medicine, National University of Singapore, Singapore 117597

An essential use of genome sequence data is to examine the individual functions of predicted ORF's within the genome and the relationships between genes at the expression level efficiently and systematically. Many existing methods such as northern blotting, RNAse protection assay and others are very limited and do not provide sufficient throughput effectively to exploit the genomic data.

DNA microarray (DNA chips), are simply glass surfaces bearing orderly arrangements of thousands of DNA fragments at discrete places, on which the fluorescent labeled DNA or RNA are ready for hybridization. This is the most powerful tool for monitoring the simultaneous expression of many thousands of genes and to larger-scale gene discovery in many different organisms (Global gene expression profiling). In the field of toxicology, the potential application of toxicogenomics to indicate the toxicity of unknown compounds has been suggested but remains largely unsubstantiated to date. A major hypothesis of toxicogenomics is that global changes in the expression of individual mRNAs (i.e., the transcriptional responses of cells to toxicants) will be sufficiently distinct, robust, and reproducible to allow discrimination of toxicants from different classes, which can act as fingerprint. Fingerprinting using microarray is a novel approach, which can be used to identify toxin function, classification of chemicals/toxins based on the pattern of altered gene expression, grouping of toxins, identifying new therapeutic drugs etc. In addition, there has not been any attempt so far to systematically define the full repertoire of toxin-regulated genes. Identification of these genes is required not only for revealing the nature of the signaling pathway/s but also for defining the set of proteins that are induced or repressed by these toxins. In our present study, we used four toxins, three of these, candoxin (reversible antagonist of muscle acetylcholine receptors from *Bungarus candidus*), tetrodotoxin (blocker of neuronal voltage-gated sodium channels from puffer fish) and toxin X (Acch esterase inhibitor), are neurotoxins. The fourth, Aflatoxin B1 (fungal toxin), is a hepatotoxin. We examined the global effects of these toxins on human cell lines (brain and liver) in a dose and time dependent approach. Using this approach, we have identified toxin targeted genes, these include genes involved in the regulation of transcription factors, oncogenes/tumor suppressor genes, signal transduction modifiers, cell growth, cell cycle, inflammation, invasion/metastasis, apoptosis, ion channels, extracellular matrix proteins, and several proteases. Gene clustering was also done with possible insight into the mechanism/s of actions of these toxins at a molecular level. Our results suggest changes in gene expression induced by these toxins can be used as fingerprints to characterize toxins. Genes identified might provide a potential drug lead or as biomarker. In addition, pathways relating to the actions of toxins as well as disease processes also could be elucidated.

Invited Lecture 3BM.2 - Hall C - 1145-1210

ABSTRACT NUMBER 23501 3BM2

DISCOVERY OF CIRCULAR PROTEIN TOXINS FROM BACTERIA, PLANTS, AND ANIMALS.

David J Craik

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Until a decade ago, circular proteins were unknown. By circular proteins we mean proteins whose N and C termini are linked in a peptide bond to form a continuous cycle of amide bonds in the peptide backbone, as distinct from proteins that are cyclic by virtue of disulfide bonds. Such circular proteins may also be distinguished from well-known cyclic peptides such as gramicidin S and cyclosporin, which are microbial products synthesized by peptide synthetases. By contrast, circular proteins are true gene products that are post-translationally modified to produce the head-to-tail cyclic backbone. Currently known circular proteins are found in bacteria, plants and animals and range in size from 14-70 amino acids. Many have toxic properties that relate to their role in host defense or the mediation of microbial competitions. By far the largest family of naturally occurring circular proteins is the plant cyclotides discovered in our laboratories over the last decade. Cyclotides are typically 30 amino acids in size and contain six cysteine residues bonded together in a cystine knot arrangement. In this unique structural motif two disulfide bonds and their connecting backbone sequence form an embedded ring in the structure that is threaded by a third disulfide bond. Cyclotides have a diverse range of biological activities ranging from uterotonic effects, antimicrobial effects, inhibition of neurotensin binding and anti-HIV effects. They also have mild haemolytic activity. Despite some of these toxic activities they have created much excitement as templates for pharmaceutical design because of their exceptional stability. For example, in native medicine applications in Africa, women boil a plant now known to contain cyclotides to make a tea that is taken during labour to accelerate uterine contractions in childbirth. Biological activity survives boiling. This presentation will describe this discovery and structural characterization of the plant cyclotides. We have also undertaken structural studies on a number of other naturally occurring circular peptides and these are compared with the cyclotides. Microcin J25 is one peptide of particular interest that was initially proposed to have a macrocyclic peptide (Blond et al 1999) but recent studies in our laboratory have shown that instead it contains a unique sidechain-to-backbone cyclisation and is in fact a lasso type structure. The C-terminal tail of the molecule threads through the embedded ring in the "lasso" to produce a remarkable noose like structure.

References

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Invited Lecture 3BM.3 - Hall C - 1210-1235

ABSTRACT NUMBER 14901 3BM3

NMR-BASED STRUCTURAL ANALYSIS OF α-NEUROTOXINS BOUND TO COGNATE PEPTIDES DERIVED FROM NICOTINIC ACETYLCHOLINE RECEPTOR SEQUENCES PROVIDES INSIGHTS INTO THE STRUCTURAL BASIS FOR TOXIN-RECEPTOR RECOGNITION.

E Hawrot

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We have used solution NMR methods to determine the structures of the stoichiometric complexes formed between α-neurotoxins (α-bungarotoxin or α-cobratoxin) and a variety of cognate peptides prepared recombinantly using sequence information from skeletal muscle (α1) or neuronal (α7) nicotinic acetylcholine receptors (e.g., Protein Data Bank Code 1LXG, 1LXH, 1IDG, 1IDH, 1KC4, 1KL8). A comparison of the free and bound forms of the a-neurotoxins reveals significant conformational rearrangements in regions previously suggested to be flexible; loops I, II, and the C-terminal tail. The bound peptides are located in a region bordered by toxin loops I and II with some contributions from the C-terminal tail. In addition, aromatic residues in the receptor-derived peptide appear to play a major role in the toxin-peptide contact zone. Several of the calculated structures suggest that cation-p interactions may be significantly involved in binding. Studies with LSIII from *Laticauda semifasciata* indicate that although this toxin is generally considered to be a long a-neurotoxin, its inability to bind receptor-derived peptides is more characteristic of the short a-neurotoxin, erabutoxin. The relatively short C-terminal tail region of LSIII appears to be responsible for this behavior. Importantly, when the NMR-derived structures of the complexes were superimposed onto the X-ray crystallographic structure of the AChBP (acetylcholine-binding protein; 1I9B; Brejc et al., 2001), a soluble homologue of the extracellular domain of the α7 nicotinic receptor, the a-neurotoxin was positioned at the peripheral surface of the inter-subunit interface in the AChBP in a manner that occludes the agonist binding site. Studies involving "footprinting" of α-neurotoxin binding to appropriately mutated nicotinic receptors expressed in HEK293 cells provide further evidence that loop C in the receptor α-subunit is the major determinant for α-neurotoxin binding. In addition, appropriate mutation of loop C receptor residues confers α-bungarotoxin sensitivity to the α3 subunit which is normally completely insensitive to this toxin. Analogous studies are now underway to introduce such "pharmatope" sequences into non-α subunits. This work was supported by NIH NS34348, GM32629, RR08240 and NSF DBI-9723282.

THURSDAY September 18th

SCIENTIFIC PROGRAMME

Plenary Lecture Session 4AH - Hall C - 0900-1030

Session Chairperson: Julian White

4AH1 David A. Warrell
MANAGEMENT OF SNAKEBITES IN TROPICAL COUNTRIES: HAS THERE BEEN ANY REAL IMPROVEMENT?

4AH2 André Ménez
DISCLOSING AND EXPLOITING THE MOLECULAR BASIS OF THE SPECIFICITY AND EVOLUTION OF FUNCTIONAL DETERMINANTS IN TOXIC MINIPROTEINS.

Poster Session 4PF.3 - Glass Foya - 1100-1140

Poster Abstracts can be found at the end of Thursday's abstracts

Invited Lecture Session 4BH - Hall C - 1140-1300 - Understanding Marine Toxins

Session Chairperson: Julian White

4BH1 Jamie Seymour
ECOLOGICAL REASONS FOR VARIATION IN VENOM LETHALITY AND SYMPTOMS IN HUMAN ENVENOMINGS FROM CNIDARIANS.

4BH2 D.J. Adams
A-CONOTOXINS: NICOTINIC ACETYLCHOLINE RECEPTOR ANTAGONISTS AS PHARMACOLOGICAL TOOLS

4BH3 Richard J. Lewis
INHIBITION OF THE NOREPINEPHRINE TRANSPORTER BY THE VENOM PEPTIDE χ-MRIA

Invited Lecture Session 4BM - Meeting Room 1&2 - 1140-1300 - Haemostasis and Toxins

Session Chairperson: Cassian Bon

4BM1 Francis S. Markland
DISINTEGRINS AND ANGIOGENESIS

4BM2 Aura S Kamiguti
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4BM3 R. Manjunatha Kini
PROTHROMBIN ACTIVATORS FROM SNAKE VENOMS

Oral Papers Session 4CH - Hall C - 1400-1530 - Marine Toxinology

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A NEW CNIDARIAN NON-CNIDOCYSTIC TOXIC PROTEIN

4CH2 Timo J. Nevalainen
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4CH4 T Turk
A CYTOLYTIC PROTEIN FROM THE ANTARCTIC HETERONEMERTINE PARBORLASIA CORRUGATUS AND ITS MODE OF ACTION

4CH5 R. Saminathan
ISOLATION, CHARACTERIZATION AND MOLECULAR SIMULATION OF TWO NOVEL OMEGA-CONOTOXINS FROM THE VENOM OF MARINE SNAIL CONUS AMADIS

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ANIMAL VENOMS: A PROTEOMIC APPROACH

Oral Papers Session 4CM - Meeting Room 1&2 - 1400-1530 - Haemostasis and Toxins

Session Chairperson: Francis Markland

4CM1 Solange M. T. Serrano
FUNCTION OF THE CYSTEINE-RICH DOMAIN OF THE HEMORRHAGIC METALLOPROTEINASE ATROLYSIN A: TARGETING ADHESION PROTEINS COLLAGEN I AND VON WILLEBRAND FACTOR.

4CM2 Md. Abu Reza
CDNA SEQUENCE OF FACTOR X OF AUSTRALIAN ROUGH SCALE SNAKE TROPIDECHIS CARINATUS AND ITS COMPARISON WITH THE VENOM PROTHROMBIN ACTIVATOR, TROCARIN D

4CM3 Liam St Pierre
COMPARATIVE STUDY OF HAEMOSTATIC FACTORS FROM THE VENOM GLAND OF THE COMMON BROWN SNAKE (PSEUDONAJA TEXTILIS) AND RELATED SPECIES.

4CM4 Yajnavalka Banerjee
ISOLATION AND CHARACTERIZATION OF ANTICOAGULANT PROTEIN FROM THE VENOM OF HEMACHATUS HAEMACHATUS

4CM5 Serrano, S. M. T.
NEW BIOLOGICAL ACTIVITY OF HF3, AN HEMORRHAGIC METALLOPROTEINASE ISOLATED FROM BOTHROPS JARARACA VENOM, ON MACROPHAGE FUNCTION.

4CM6 M Bunc
THROMBOCYTE AGGREGATION CAUSED BY HEAD-TO-TAIL 3-ALKYLPYRIDINIUM POLYMERS ISOLATED FROM THE MARINE SPONGE RENIERA SARAI IS PARTLY BLOCKED BY THE COMBINATION OF ACETYL SALICIC ACID, CLOPIDOGREL AND ABCIXIMAB

Oral Papers Session 4CR - Meeting Room 3 - 1400-1530 - Clinical Toxinology

Session Chairperson: Ken Winkel

4CR1 J.White
CLINICAL TOXINOLOGY - WHERE TO NOW?

4CR2 GK Isbister
PROSPECTIVE STUDY OF DEFINITE CATERPILLAR EXPOSURES IN AUSTRALIA

4CR3 David Williams
EPIDEMIOLOGY OF SNAKEBITE IN THE MEKEO REGION OF CENTRAL PROVINCE, PAPUA NEW GUINEA

4CR4 G.K. Isbister
AUSTRALIAN MYGALOMORPH SPIDER BITE, INCLUDING FUNNEL WEB SPIDERS (ATRACINAE) AND MOUSE SPIDERS (MISSULENA SPP).

4CR5 Little M
FIRST AID FOR POTENTIALLY LETHAL JELLYFISH STINGS IN AUSTRALIA

4CR6 M.Iqbal Alam
SNAKE VENOM NEUTRALIZATION BY INDIAN MEDICINAL PLANTS (VITEX NEGUNDO AND EMBLICA OFFICINALIS) ROOT EXTRACTS.

Oral Papers Session 4DH - Hall C - 1600-1730 - Toxins as Tools

Session Chairperson: Graham Nicholson

4DH1 D Butera
CLONING, EXPRESSION AND CHARACTERIZATION OF A BI-FUNCTIONAL DISINTEGRIN/ALKALINE PHOSPHATASE HYBRID PROTEIN

4DH2 Frédéric A. Meunier
GLYCEROTOXIN FROM GLYCERA CONVOLUTA STIMULATES NEUROSECRETION BY UP-REGULATING N-TYPE CA2+ CHANNEL ACTIVITY

4DH3 D Suput
ACHE - INHIBITORY PSEUDOZOANTHOXANTIN-LIKE COMPOUND FROM PARAZOANTHUS AXINELLAE ADRIATICUS

4DH4 L. Fishman
NEUROTOXIC PEPTIDE TRANSLOCATION THROUGH AN INSECT GUT.

ISTH Meeting 4DM - Meeting Room 1&2 - 1400-1530 - Haemostasis and Toxins

Session Chairperson: Cassian Bon & M Kini

This session is officially for the International Society for Thrombosis and Haemostasis expert committee on toxins affecting haemostasis, but may include some papers, details not available at time of printing the Abstract Book.

Oral Papers Session 4DR - Meeting Room 3 - 1400-1530 - Clinical Toxinology

Session Chairperson: David Warrell

4DR1 Kenneth D Winkel
THE CARDIOVASCULAR ACTIONS OF THE IRUKANDJI (CARUKIA BARNESI) JELLYFISH

4DR2 Little M
IRUKANDJI SYNDROME: MORE THAN ONE JELLYFISH (PART 1)

4DR3 Seymour J
IRUKANDJI SYNDROME: MORE THAN ONE JELLYFISH (PART 2)

4DR4 S Ramasamy
IN VIVO EXAMINATION OF TREATMENT STRATEGIES FOR BOX JELLYFISH (CHIRONEX FLECKERI)

Official Business Meeting of the IST - Hall C - 1700-1800

Session Chairperson: Herve Rochat, Dietrich Mebs

This important meeting of the IST is only held once every 3 years, at the IST World Congress. It is here that directions for future congresses are discussed, thus it affects all IST members and all should endeavour to attend. Groups considering offering to host future IST Congresses should prepare and present their case at this meeting. Other matters to be discussed include IST Membership Fees, TOXICON and the next set of IST Office Bearers.

Congress Dinner - Hall F - 1900-late

This Official Dinner is included in registration fees, except for reduced rate (student) fees. This latter group are most welcome to attend; the cost will be Aus$80. Numbers must be notified in advance.

THURSDAY September 18th

Plenary Lecture 4AH.1 - Hall C - 0900-0950

ABSTRACT NUMBER 05401 4AH1

MANAGEMENT OF SNAKEBITES IN TROPICAL COUNTRIES: HAS THERE BEEN ANY REAL IMPROVEMENT?

David A. Warrell

Head, Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU, UK

The burden of human suffering: Population based studies of snakebite in Nigeria, Senegal, Kenya, West Bengal and Nepal have confirmed a high snakebite mortality. For every fatality, several survivors are left with permanent physical handicap resulting from the necrotic local effects of the venom or from complications of traditional treatments. Most victims are children or previously healthy young agricultural workers. The impact of deaths and disability in these groups is specially damaging to the rural communities and to national food production.

Neglect: The chronic neglect by Western Medicine of this environmental health hazard of the rural tropics is difficult to reconcile with the great scientific interest in snake venoms and popular fascination with the snakes themselves.

Advances in treatment of snakebite: The scientific study of snakebite treatments started with Felice Fontana in the late 18th century. The only real advances in management since then have been the use of antivenoms, pressure-immobilisation for the first-aid of elapid envenoming and application of advances in the medical treatment of organ and tissue failure to the resuscitation of snakebite victims: (1) assisted ventilation for respiratory paralysis, (2) dialysis or haemoperfusion for acute renal failure, (3) circulating volume replacement and vasopressor drugs for shock and (4) antibiotics for secondary infections.

Situation in tropical countries: In tropical developing countries the greatest challenges are to apply appropriate first-aid treatment and to transport the patient to a place where they can receive medical treatment as soon as possible, while delaying the progression of systemic envenoming or preventing death from paralysis or shock and the evolution of local necrosis. Community education in first-aid and the training of medical ancillaries or doctors in techniques of resuscitation and assisted ventilation are the key elements in these early stages of teatment. Algorithmic approach to treatment: In hospital or dispensary, simple algorithms may help to guide treatment but antivenom is usually crucial to survival if the patient is severely envenomed. Lack of antivenom in many hospitals makes it necessary to recommend methods of purely conservative management such as blood transfusion for haemostatic disturbances and prolonged mechanical ventilation for neurotoxic envenoming. Inadequate supplies of safe, effective antivenom and lack of training and appropriate guidelines are the main impediments to successful treatment in the rural tropics.

New approaches to treatment: Promising experimental approaches include injection at the bite site of chemical inhibitors of venom components (enzymes), such as metalloproteinases, responsible for local necrosis; and the design of immunoglobulin fractions and delivery systems for use in first aid.

The future: More effort should be devoted to applying the considerable knowledge of venom activity and pathophysiological changes in envenomed animals to improving the treatment of human snakebite victims.

Plenary Lecture 4AH.2 - Hall C - 0950-1030

ABSTRACT NUMBER 00301 4AH2

DISCLOSING AND EXPLOITING THE MOLECULAR BASIS OF THE SPECIFICITY AND EVOLUTION OF FUNCTIONAL DETERMINANTS IN TOXIC MINIPROTEINS.

André Ménez*, Claudio Vita, Sylvaine Gasparini & Denis Servent.

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Toxins are often small proteins rich in disulphide bonds. The biological action of these miniproteins depends upon a specific and often tight binding to a target, a step that is either directly responsible for the blockade of the target's function or which precedes other, often more complex, steps that lead to a modulation of the target's activity. The parameters dictating the specificity and possibly the evolutionary trend of the binding determinants of toxic miniproteins will be presented. It will also been shown that the acquired knowledge can be exploited to design and engineer novel toxic-like miniproteins with predetermined complex activities.

More precisely, this presentation will describe (1) a variety of folds displayed by toxic miniproteins; (2) the general properties of their binding determinants; (3) molecular features of the binding determinants that provide toxic miniproteins with given specificity profiles or, more generally, with given pleiotropic properties i.e. how a toxic miniprotein can block various target subtypes and conversely how a target can be blocked by various toxic miniproteins, and (4) the features that suggest that a binding determinant may drift from one specificity to another.

On the basis of the above observations, we have replaced the determinant naturally "used" by a toxic miniprotein to block a voltage-gated K^+ channel by a new one. This newly introduced surface nicely mimics the site by which the human CD4 not only blocks the membrane protein gp120 from HIV but also triggers the unmasking of the site by which gp120 binds to neutralizing antibodies. Our results demonstrate that the folds of toxic miniproteins can display more complex binding determinants than observed in nature. They also show that these folds may be useful in engineering new drug-like substances.

Invited Lecture 4BH.1 - Hall C - 1140-1210

ABSTRACT NUMBER 24601 4BH1

ECOLOGICAL REASONS FOR VARIATION IN VENOM LETHALITY AND SYMPTOMS IN HUMAN ENVENOMINGS FROM CNIDARIANS.

Jamie Seymour

School of Tropical Biology, James Cook University, Cairns

Toxinology studies of cnidarians have predominantly been focused around medical areas of research, and little if any literature exists which investigates the ecological reasons behind why some of these venoms are lethal to humans as well as producing distinctly different symptoms in envenomed victims. The few studies attempting to elucidate the ecological reasons behind these differences in the cnidarians have been restricted to areas of decreasing predation. However, when other ecological areas are investigated, such as feeding ecology, the reasons for variations in venom lethalities and symptoms caused by cnidarians, especially cubozoans (box jellyfish) appears to be more ecologically sound then the predator theory.

In human envenomings by box jellyfish, two types of stings are recognised, type A and type B. Type B envenomings show rapid onset of symptoms while there may be a delay of some 20-30 minutes in type A stings. For box jellyfish that cause type B stings, data suggest that their nematocyst shafts are perforated along the length allowing venom to flow from these holes. In contrast, species that cause type A stings have entire shafts, resulting in venom exiting the shaft only at the distal end. The structural differences in the nematocyst shafts between species causing type A and type B stings results in venom being deposited into interstitial cellular spaces (type A stings) or directly into the circulatory system (type B stings). This may then result in a time delay for type A (Irukandji like syndrome) symptoms but give immediate symptoms in type B (eg *Chironex fleckeri*) type envenomings. However, the differences in the structure of these shafts of the nematocysts used by these two groups, appears to be related to prey type. Those causing Type A stings feed predominantly on larval fish and deposit venom directly into the lymph system (which has direct and continuous connection with the circulatory system) of the prey. Those species causing type B stings feed primarily on fish and deposit venom directly into the circulatory system.

Investigations into the feeding ecology of two closely related cubozoans, namely *Chironex fleckeri* (lethal to humans) and *Chiropsalmus* sp. (non lethal to humans), show that lethality to humans also varies with prey type. Nematocysts, (the apparatus involved in venom delivery), of these two species show a striking variation. In *Chiropsalmus* sp., a species that feeds exclusively on shrimp, there are no changes in the ratio of the three different types of nematocyst present in the tentacles of this species. In contrast, for *Chironex fleckeri*, the ratio of different types of nematocysts varies with size. In small animals the ratios similar to that of *Chiropsalmus* sp., however, with an increase in body size in *C. fleckeri*, the nematocyst ratio changed, with mastigophores (nematocysts that hold the lethal venom component for prey) increased significantly in proportion to the other types. These changes correlated with a change in the prey of *C. fleckeri* with larger animals shifting from a predominantly shrimp (invertebrate) based food source to that of predominantly fish (vertebrates).

These results suggest that feeding ecology plays a major role in determining the type of venom, its lethality and the symptoms seen in envenomed humans. Consequently, ecological studies on feeding ecology in venomous marine animals are liable to isolate species that may present a threat for human envenomations indicating which species need an immediate research focus. This is preferable to waiting for incidences of human mortality to occur before investigations begin and research is prioritised.

Invited Lecture 4BH.2 - Hall C - 1210-1235

ABSTRACT NUMBER 24001 4BH2

α-CONOTOXINS: NICOTINIC ACETYLCHOLINE RECEPTOR ANTAGONISTS AS PHARMACOLOGICAL TOOLS

D.J. Adams[1], M. Loughnan[2], A. Nicke[1,2], P.F. Alewood[2] and R.J. Lewis[1,2].

[1]*School of Biomedical Sciences and* [2]*Institute for Molecular Biosciences, University of Queensland, Brisbane, QLD 4072 Australia.*

Conotoxins are peptides expressed in the venom ducts of marine snails of the genus *Conus* and have been divided into a number of major classes based on their pharmacological activity and cysteine frameworks. Conotoxins provide a vast library of peptides (>500,000 estimated) with unique abilities to discriminate among types and subtypes of ion channels and receptors. The α-conotoxins specifically antagonize nicotinic acetylcholine receptor (nAChRs) and new α-conotoxins have been discovered, which have the potential to distinguish among the nAChR subtypes expressed in neurons. The α-conotoxins contain 4 cysteines, which in their natural conformation form disulphide bridges giving the molecule a globular two-loop configuration with side-chains projecting from a rigid backbone. The α-conotoxin PnIA, isolated from *C. pennaceus*, is 16 amino acids long and has been demonstrated to be competitive inhibitor of native and recombinant nAChRs, with poor selectivity between receptor subtypes. A leucine for alanine substitution at position 10 makes the toxin a highly selective inhibitor of the α7 nAChR subtype. NMR studies have shown that the A10L substitution in PnIA does not affect the backbone structure of the molecule or angle of the projecting side-chains, therefore the increase in potency must be attributed to the longer aliphatic side-chain of leucine 10. Using assay-directed fractionation of *C. geographus* crude venom, a novel α-conotoxin GID with an extended N-terminal tail and an arginine at position 12 that contributes to α4β2 nAChR subtype selectivity has been isolated and characterized. Deletion of the N-terminal sequence (GIDΔ1-4) significantly decreased activity at the α4β2 nAChR but did not affect potency at α3β2 or α7 nAChRs. Comparison of GID with other 4/7 α-conotoxins which possess an NN(P/O) motif in loop II, revealed a correlation between increasing length of the aliphatic side-chain in position 10 and greater a7 versus α3β2 selectivity. There is a growing list of post-translational modifications including C-terminal amidation generally, glutamic acid γ-carboxylation (eg. α-GID), and tyrosine sulfation that contribute to the diversity of α-conotoxins. An analytical approach using combined LC/MS techniques and a functional assay identified novel sulfated α-conotoxins, AnIA, AnIB and AnIC, from *C. anemone*. The most active of these, α-AnIB, was further characterized and used to investigate the influence of post-translational modifications on affinity. Synthetic AnIB exhibited subnanomolar potency at the α3β2 nAChR and was 200-fold less active on the α7 nAChR. The unsulfated peptide [Tyr16]AnIB exhibited a two-fold and ten-fold decrease in α3β2 and α7 activity, respectively. Comparison with similar 4/7 α-conotoxin sequences suggests that glycine 11 and glutamine 14 constitute important determinants for α3β2 selectivity, whereas the C-terminus and sulfation at tyrosine 16 influence α7 affinity. Our studies provide a basis for understanding the structural features which confer α-conotoxin selectivity for neuronal nAChR subtypes

Invited Lecture 4BH.3 - Hall C - 1235-1300

ABSTRACT NUMBER 24501 4BH3

INHIBITION OF THE NOREPINEPHRINE TRANSPORTER BY THE VENOM PEPTIDE χ-MRIA

Iain A. Sharpe†§, Elka Palant¶, Christina I. Schroeder†, David M. Kayell, David J. Adams§, Paul F. Alewood†, Lesley J. Bryan-Lluka§, Heinz Bönisch§** and **Richard J. Lewis†¶.**

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χ-Conopeptide MrIA (χ-MrIA) from the venom of the predatory marine snail *Conus marmoreus* selectively inhibits the norepinephrine transporter (NET). The binding of the NET inhibitors, [3H]-nisoxetine and [3H]-mazindol, to the expressed rat and human NET was inhibited by χ-MrIA in a competitive manner. It has previously been demonstrated that χ-MrIA does not compete with norepinephrine, unlike classically described NET inhibitors, such as nisoxetine and mazindol. This pattern of behaviour implies that the binding site for χ-MrIA overlaps the antidepressant binding site and is wholly distinct from the substrate binding site. The nature of the interactions of χ-MrIA with human NET were investigated by determining the effects of NET point mutations on the inhibitory potency of χ-MrIA. Of 18 mutations where NET amino acid residues were exchanged to those of DAT, MrIA had increased potency for inhibition of [3H]norepinephrine uptake at three (in predicted extracellular loops 3 and 4 and transmembrane domain (TMD) 8), and decreased potency at one mutation (in TMD6 and intracellular loop (IL) 3). Of 12 additional mutations in TMDs 2, 4, 5 and 11 and IL1, three mutations (in TMD2 and IL1) had reduced χ-MrIA inhibitory potency. The inhibitory effect of χ-MrIA was found to be dependent on Na^+, with the conopeptide becoming a less effective blocker of [3H]-norepinephrine by the NET under conditions of reduced extracellular Na^+. In this respect, χ-MrIA is similar to the antidepressant inhibitors of the NET. These data indicate that χ-MrIA binding to hNET is at a site distinct from norepinephrine, but overlapping with the binding sites for tricyclic antidepressants and cocaine. The structure-activity relationship of χ-MrIA was investigated by Ala scanning. Four residues in the first cysteine-bracketed loop of χ-MrIA, and a His in loop 2 played a dominant role in the interaction between MrIA and NET. Ha chemical shift comparisons indicated that side-chain interactions at these key positions were structurally perturbed by replacement of Gly6.

Invited Lecture 4BM.1 - Meeting Room 1&2 - 1140-1210

ABSTRACT NUMBER 05801 4BM1

DISINTEGRINS AND ANGIOGENESIS

Francis S. Markland

University of Southern California, Keck School of Medicine

For a tumor to grow bigger than 1-2 cu. mm., it requires a vascular supply. The process of supplying blood to a growing tumor is called angiogenesis or neovascularization. The blood supply provides nutrients and growth factors for the tumor, and importantly serves as an escape route for the tumor cells to disseminate or metastasize.

We have been studying an interesting protein from southern copperhead snake venom that is a member of the venom disintegrin family. Disintegrins are small peptides with a number of intrachain disulfide bonds; they contain an Arg-Gly-Asp (RGD) sequence, or a closely related sequence, that is critical for binding to integrins on the surface of cells. Integrins are heterodimeric proteins that are involved in cell-cell and cell-extracellular matrix (ECM) communication. Newly growing endothelial cells, as well as a number of different types of cancer, express integrin αvβ3 (the vitronectin receptor).

The disintegrin from southern copperhead venom is called contortrostatin in honor of the snake species from which it is derived (*Agkistrodon controtrix contortrix*), as well as the fact that it blocks platelet aggregation (statin activity). In fact, disintegrins were first reported as platelet aggregation inhibitors.

Contortrostatin is unusual among the disintegrin family in that it is a homodimer and the first homodimeric disintegrin to be reported. Contortrostatin has a molecular weight of 13,500 and its two chains are held together by disulfide bond(s). Its homodimeric structure is critical to at least part of its biological activity.

My laboratory has focused on the antiangiogenic properties of the protein. Because of the ability of this protein to bind to several different integrins on the surface of cancer and endothelial cells, it is a very potent anticancer agent. We have now looked at four different forms of cancer in animal models (breast, prostate, ovarian cancers and glioma, a deadly brain cancer) and in all cases the venom protein is a very effective inhibitor of tumor growth and dissemination. We have strong evidence that at least a significant part of this anticancer activity is due to its antiangiogenic activity.

Studies are now continuing on a mechanism to deliver the protein and target it to the tumor site. This would avoid potential side effects of the protein, and its recognition by the immune system. These studies have produced some surprising and exciting results and suggest that there may be translational potential for this protein as an antitumor agent.

Invited Lecture 4BM.2 - Meeting Room 1&2 - 1210-1235

ABSTRACT NUMBER 06001 4BM2

PLATELETS AND COLLAGEN INTERACTIONS: MORE TO LEARN WITH P-III SNAKE VENOM METALLOPROTEINASES?

Aura S Kamiguti [1], Paul Gallagher [2], Cezary Marcinkiewcz [3], David Theakston [4], Mirko Zuzel [1] and Jay W. Fox [2]

1 Department of Haematology, University of Liverpool, Liverpool, UK; 2 Department of Microbiology, University of Virginia, Charlottesville, USA; 3 Temple University, Department of Biology, Philadelphia, USA; 4 Liverpool School of Tropical Medicine, Liverpool, UK.

The interaction of platelets with subendothelial collagen during vascular injury plays a major role in haemostasis. Platelets adhere to collagen fibres through specific membrane receptors $\alpha 2\beta 1$ integrin and glycoprotein VI. Among different snake venom toxins that inhibit this interaction, the class P-III snake venom metalloproteinases (SVMPs) are unique. The soluble P-III SVMPs are characterised by an N-terminal Zn^{++}-dependent catalytic domain, a non-RGD-containing disintegrin-like domain and a C-terminal cysteine-rich region. These enzymes are structurally homologous to the cell membrane-bound enzymes ADAMs, but unlike these, they are haemorrhagic. Jararhagin from *Bothrops jararaca* venom and Atrolysin A from *Crotalus atrox* venom belong to the P-III class group. Native or enzymatically inactive Jararhagin abolishes platelet aggregation with collagen, indicating that the catalytic site does not mediate the inhibitory activity. However, in the catalytic domain, a site containing the sequence RKKH inhibits collagen/platelet interactions. The homology of the disintegrin-like domain with viper RGD disintegrins is also remarkable, except that often an SECD sequence instead is found in the P-III SVMPs and this region also displays similar inhibitory effect. Recent work has also shown that the cysteine-rich domain per se can inhibit the responses of platelets to collagen. We have identified in both Jararhagin and Atrolysin A, two homologous peptide sequences in the cysteine-rich regions that display such an activity. However, although the cysteine-rich-based peptides inhibit aggregation and adhesion exclusively via $\alpha 2\beta 1$ integrin, signals generated by platelet/collagen interaction (i.e., Syk phosphorylation) in the presence of these peptides are preserved most likely due to intact glycoprotein VI. Furthermore, the Jararhagin-based cysteine-rich peptides alone enhance platelet protein tyrosine phosphorylation, demonstrating that upon interaction, the peptides may have an activating effect but, unlike collagen, do not lead to platelet aggregation. It appears therefore that for full platelet responses to collagen both $\alpha 2\beta 1$ integrin and glycoprotein VI are necessary. These data not only acknowledge the complexity of these enzymes with multifunctional domains but also may contribute to the understanding of signalling pathway underlying platelet interaction with collagen.

Invited Lecture 4BM.3 - Meeting Room 1&2 - 1210-1300

ABSTRACT NUMBER 06601 4BM3

PROTHROMBIN ACTIVATORS FROM SNAKE VENOMS

R. Manjunatha Kini

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Thrombin formation from its inactive zymogen prothrombin is a key step in the blood coagulation cascade. Several procoagulant factors that target this crucial step in the cascade and activate prothrombin have been isolated from snake venoms. These prothrombin activators are classified into four groups based on their structural and functional characteristics. Groups A and B are metalloproteinases, whereas groups C and D are serine proteinases. Earlier we had shown that group D prothrombin activators are structurally and functionally similar to mammalian coagulation factor Xa. Our recent studies have shown that group C prothrombin activators are similar to mammalian coagulation factor Xa-factor Va complex. Here, the structural features of the group C and D prothrombin activators will be compared with that of their mammalian counterparts. The implications of the observed differences to the structure-function relationships of the snake venom prothrombin activators in terms of the prothrombinase complex formation and toxicity will be described.

1. J. S. Joseph, M. C. M. Chung, K. Jeyaseelan and R. M. Kini, Blood, 94, 621-631, 1999.
2. V. S. Rao, J. S. Joseph and R. M. Kini, Biochem. J. 369, 635-642, 2003.
3. V. S. Rao and R. M. Kini, Thrombos. Haemos. 88, 611-619, 2002.
4. V. S. Rao, S. Swarup and R. M. Kini, Blood, in press, 2003.

Marine Toxinology - Hall C - 1400-1415

ABSTRACT NUMBER 18101 4CH1

A NEW CNIDARIAN NON-CNIDOCYSTIC TOXIC PROTEIN

Daniel Sher [1,2], Yelena Fishman [1], Mingliang Zhang [1,3], and Eliahu Zlotkin [1,2]

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Similar to other animal venom systems, the toxic chemistry of cnidarians is based on a wide array of cytolytic, pore-forming, phospholypatic and neurotoxic polypeptides. Due to their paralytic action, the storage and delivery sites of cnidarian toxins are axiomatically attributed to the ectodermal stinging cells (cnidocysts) which are discharged for either offensive (prey capture) and/or defensive purposes.

In the green hydra, *Chlorohydra viridissima*, a homogenate of the whole body reveals toxicity that is clearly different from the toxicity found in isolated cnidocysts. Using anion-exchange chromatography, a major paralysis-inducing (neurotoxic) factor has been isolated and purified from the body homogenate, resulting in a 27kDa protein with a pI of 7.2. This protein, which we named Hydralysin , was cloned and functionally expressed in *E. coli*. Hydralysin is unique in that:

1) Western blotting has revealed that Hydralysin is derived from non-cnidocystic origin.

2) Hydralysin reveals a clear animal-group-selective toxicity, being at least an order of magnitude more toxic to arthropods than to vertebrates.

3) Hydralysin possesses a novel primary structure, possibly revealing a subtle homology to pore-forming toxins from bacteria and mushrooms.

4) Hydralysin has a fast cytolytic effect on insect sf9 cells but not on human HEK-293 cells.

The above data raise the question of the biological role played by a toxic protein which is devoid of a mechanical delivery system (non-cnidocystic), but, on the other hand, is able to affect cell membranes.

We can offer two major, either alternative or joint, hypotheses: Firstly, Hydralysin serves as an alomone in order to deter natural enemies (predators, pathogens) and/or to prolong the paralysis of an ingested prey in the gastrovascular cavity. Secondly, it may fulfill an endogenous regulatory role in the hydra's physiology.

Marine Toxinology - Hall C - 1415-1430

ABSTRACT NUMBER 17001 4CH2

PHOSPHOLIPASE A_2 IN CNIDARIA

Timo J. Nevalainen

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Phospholipase A_2 (PLA_2) catalyzes the hydrolysis of the sn-2 acyl bond of glycerophospholipids to generate free fatty acids and lysophosholipids. Snake venom and mammalian secretory PLA_2s are calcium dependent enzymes that play important roles in phospholipid metabolism, inflammation, host defense and digestion. Distinct PLA_2s have been characterized in a number animal species including marine invertebrates. Cnidaria comprise a phylum of aquatic animals including sea anemones, corals and jellyfishes.

In the current study, varying levels of PLA_2 catalytic activity were detected in tissue homogenates of specimens representing the classes Anthozoa, Hydrozoa, Scyphozoa and Cubozoa of the phylum Cnidaria. The highest PLA_2 levels were measured in the stony coral *Pocillopora damicornis* and the hydrozoan fire coral *Millepora* sp. that cause skin irritation upon contact. High levels of PLA_2 activity were found in the acontia of the sea anemones *Metridium senile* and *Adamsia carciniopados*. Acontia are long threads containing nematocysts and used in defense and aggression by the animals. Tentacles of scyphozoan and cubozoan species had high levels of PLA_2 activity. The tentacles of the jellyfish *Cyanea capillata* that cause skin irritation and those of the box jellyfish *Chironex fleckeri*, which may inflict lethal stings, had high PLA_2 activity. The functions of cnidarian PLA_2s may include roles in the capture and digestion of pray and defense of the animal.

Marine Toxinology - Hall C - 1430-1445

ABSTRACT NUMBER 4CH3

CIGUATERA, A TROPICAL FISH POISONING

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Ciguatera is a pleomorphic syndrome consisting of a range of gastrointestinal, neurological and cardiovascular signs and symptoms that follow the consumption of warm-water marine fish contaminated with sodium channel activator toxins known as ciguatoxins. The disease is rarely fatal and its severity and duration may be reduced with intravenous mannitol. At least two structurally distinct families of ciguatoxins, one from the Pacific and one from the Caribbean, have been identified. A third family of ciguatoxins has recently been identified in ciguateric reef fishes of the Indian Ocean. All these ciguatoxins (CTX) most likely arise from certain strains of the benthic dinoflagellate, *Gambierdiscus toxicus*. Following blooms of *G. toxicus*, these toxins accumulate in fish through marine food chains to levels that affect human health. Factor(s) influencing such bloom formation are unclear. P-CTX-1, the most potent sodium channel toxin known, is the major toxin contributing to ciguatera caused by carnivorous fish in the Pacific, causing human poisoning at levels of 0.1 ppb and above. The mouse assay is presently used to assess levels of ciguatoxin in fish extracts. *In vitro* cell-based assay, which measure the effects of ciguatoxin-induced sodium channel opening or the inhibition of [^{3}H]-brevetoxin binding, are more sensitive than *in vivo* methods and have the potential to replace the mouse assay. Analytical detection methods, in particular HPLC/tandem ionspray mass spectrometry, are also under development. Antibody-based assays offer hope for as cost-effective screens for ciguateric fish, but presently appear to lack the necessary specificity and sensitivity. A major advance in the management of ciguatera will come when a validated screen is commercially available.

Marine Toxinology - Hall C - 1445-1500

ABSTRACT NUMBER 08901 4CH4

A CYTOLYTIC PROTEIN FROM THE ANTARCTIC HETERONEMERTINE PARBORLASIA CORRUGATUS AND ITS MODE OF ACTION

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From freeze-dried mucus of the Antarctic nemertine *Parborlasia corrugatus* we have isolated 10.3 kDa basic (pI > 9.0) cytolytic protein, referred to as parborlysin. Although the purified protein sample was homogeneous by reversed phase HPLC chromatography profiles and several electrophoretic techniques, N-terminal sequence and mass spectrometric analyses revealed that it consisted of few very similar isotoxins. The N-terminal sequence of the parborlysin sample shows a high degree of homology with the sequence of cytolysin A-III from the heteronemertine *Cerebratulus lacteus*. Parborlysin in micromolar concentration range disrupts mammalian erythrocytes with an apparent detergent mode of action. Hemolytic activity was inhibited by preincubation of parborlysin with pure phosphatidic acid or with rather high concentrations of small unilamellar vesicles composed of phosphatidylcholine (PC)/phosphatidylglycerol, PC/phosphatidylinositol, and PC/phosphatidylserine. Osmotic protectants as large as 3000 Da failed to protect red cells from lysis induced by parborlysin. Further structural and pharmacological analysis of the heteronemertine cytolysins may provide new insights regarding the mechanisms by which some water soluble proteins are able to penetrate into lipid membranes and form pores or, acting as detergents, disrupt their normal structure and function.

Marine Toxinology - Hall C - 1500-1515

ABSTRACT NUMBER 17401 4CH5

ISOLATION, CHARACTERIZATION AND MOLECULAR SIMULATION OF TWO NOVEL OMEGA-CONOTOXINS FROM THE VENOM OF MARINE SNAIL CONUS AMADIS

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Research on *Conus* venom peptides (viz., ω-Conotoxin MVIIA/Ziconotide/PrialtTM) have reached the stage of treating many patients who are suffering from chronic neuropathic pain or ischemia of brain in stroke. Subsequently, some other *Conus* peptides are also in clinical trial Phase II/III to treat intractable pain experienced by terminally ill cancer and AIDS patients in situations where morphine is ineffective. There are about 18 omega-conotoxin peptides that have been identified so far from various *Conus* species, all targets neuronal Ca^{2+} channels with varying sub-type (N, L, P and P/Q) selectivity. We report here two new omega-conotoxin peptides from the venom of *Conus amadis* with following sequence: CX6CX6CCX3CX3CX2-OH (ω-conotoxin AmVIIA) and CX6CX6CCX3CX3CX2-OH (w-conotoxin AmVIIB), where 'X' represents the number of amino acid residues and 'C' indicates cysteines of omega-conotoxin motif. These two peptides were isolated and characterized by consecutive chromatographic purifications, mass spectrometric analysis and N-terminal sequencing. The biological assay for target identification and validation is being undertaken in parallel effort with in silico assay. For that, the peptides' secondary structural elements such as helix, strands and turns were determined by *ab initio* using predict-protein's standard algorithms. The molecular models of these two w-conotoxin peptides (AmVIIA and AmVIIB) were generated by using the coordinates of NMR structure of ω-conotoxin TxVII (PDB Code 1F3K) and homology modeling programme MODELLER6v2. The models of the AmVIIA and AmVIIB are optimized until that best satisfies the spatial restraints obtained. The homology-derived and stereo-chemical restraints are combined into an objective function.

Marine Toxinology - Hall C - 1515-1530

ABSTRACT NUMBER 15501 4CH6

ANIMAL VENOMS: A PROTEOMIC APPROACH

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Does mass spectrometry challenge the depicting all the peptides secreted by a venom gland ("Venome")? We evaluated analytical methods for which the maximum of individual masses can be depicted (venom mass fingerprinting). These methods mandatory include a chromatographic step in order to beat the venom complexity. The first named "off-line LC/MALDI-TOF-MS" is the combination of a classical venom HPLC (reverse phase) purification step followed by a systematic mass analysis of each chromatographic fraction by spotting them on a MALDI plate. The second named "on-line LC/ESI-MS" is a method for which the HPLC flow-through is subjected to electrospray ionisation and analysed straightaway on the mass spectrometer.

As a proof of concept, the venom of *Tityus serrulatus* (Scopiones, Buthidae) was initially subjected to a gel filtration on Sephadex G50 in order to eliminate non-toxic high-molecular-weight materials leading to two main toxic fractions (FII and FIII, displaying 90% and 10% of the venom lethality, respectively). The molecular masses in FII were predominantly between 6500 and 7500 Da corresponding to long-chain toxins that mainly act on voltage-gated sodium channels. Fraction FIII is more complex with masses displayed between 2500 and 5000 Da corresponding to short-chain toxins, most of which acting on potassium channels, and numerous other unknown peptides. After data mining, the off-line approach allowed us to increase by a factor of three the number of molecular masses that had been detected by direct MALDI-TOF-MS or LC/ESI-MS with a total of 380 different compounds (FII and FIII). There is however potential traps coupling LC to MALDI-TOF-MS such as non-specific interactions with the reverse phase matrix, degradation and cleavage of proteins, missed cleavage sites from precursors and isoforms, isomasses, leading to an overestimation. Also, MALDI-TOF-MS may be adequately used to assess venom variability due to different rates in protein expression and/or processing. Important variations were observed in venoms of a single *Tityus serrulatus* specimen extracted at different times, especially in latter extraction events. However, comparing the first extraction events — after 20 days of starvation — from ten scorpions showed small variations in relative content.

Establishing "Venomes" will undoubtedly contribute to the identification of new bioactive peptides in what we can name "natural peptide libraries".

Haemostasis & Toxins - Meeting Room 1&2 - 1400-1415

ABSTRACT NUMBER 07401 4CM1

FUNCTION OF THE CYSTEINE-RICH DOMAIN OF THE HEMORRHAGIC METALLOPROTEINASE ATROLYSIN A: TARGETING ADHESION PROTEINS COLLAGEN I AND VON WILLEBRAND FACTOR.

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The distinctive feature of Viperidae snake envenomation is the pronounced local hemorrhage resulted from the synergistic action of several toxins. Viperidae venoms are a rich source of hemorrhagic toxins characterized as zinc metalloproteinases, which are members of the Reprolysin subfamily of metalloproteinases. Toxins classified in the P-III class of reprolysins have, in addition to the proteinase domain, a disintegrin-like and a cysteine-rich domain. It has been shown that the nonproteinase domains of the P-III class of hemorrhagic toxins inhibit platelet aggregation by blocking integrin alpha2beta1 on platelets. We recently demonstrated that the recombinant cysteine-rich domain of atrolysin A is able to inhibit collagen-stimulated platelet aggregation and to interact with MG-63 osteosarcoma cells via integrin alpha2beta1 to inhibit adhesion to collagen I. In this study we show by solid-phase binding assay that native atrolysin A, and its recombinant disintegrin-like/cysteine-rich (A/DC) and cysteine-rich (A/C) domains can bind to collagen I. Interestingly, the incubation of collagen I-coated plates with recombinant A/C did not prevent the adhesion of MG-63 cells to collagen. This suggests that the cysteine rich domain can bind to both collagen I and alpha2beta1 and that collagen I contains different binding sites for the integrin alpha2beta1 and the cysteine-rich domain of atrolysin A. Kinetic constants of the interaction of A/C as well as of other metalloproteinases domains (A/DC, atrolysin A, jararhagin and catrocollastatin C) to collagen I were determined by surface plasmon resonance. We also demonstrated by solid-phase binding assay that both A/DC and A/C domains can function as von Willebrand factor binding proteins. Taken together, these studies indicate a multi-functional role for the cysteine-rich domains of class P-III metalloproteinases in the coagulopathies associated with crotalid and viperid envenoming.

Haemostasis & Toxins - Meeting Room 1&2 - 1415-1430

ABSTRACT NUMBER 18801 4CM2

CDNA SEQUENCE OF FACTOR X OF AUSTRALIAN ROUGH SCALE SNAKE TROPIDECHIS CARINATUS AND ITS COMPARISON WITH THE VENOM PROTHROMBIN ACTIVATOR, TROCARIN D

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Snake venom toxins affect several physiological systems including blood coagulation. In blood coagulation cascade the activation of prothrombin to thrombin is the key reaction and many venom procoagulants act on this specific and crucial step. Based on the functional properties and cofactor requirements for optimal activation, prothrombin activators (PAs) are classified into four groups, namely Group A, B, C and D. Recently, we have shown that Group D PAs are structurally and functionally similar to human blood coagulation factor Xa. Trocarin D from *Tropidechis carinatus* and hopsarin D from *Hoplocephalus stephensi* venoms show same domain architecture and similar biological activity as mammalian FXa. So, these snakes produce two similar proteins: one in their venom glands which act as toxins and the other in their liver which acts as a hemostatic factor. Here we report the cDNA sequence encoding the blood coagulation FX of *T. carinatus*. We isolated and sequenced the cDNA encoding FX from the liver tissue of *T. carinatus*. Deduced FX sequence shows ~69% identity with trocarin D and ~50% identity with mammalian FXa. These studies confirm the presence of two separate genes – one each for FX and trocarin D, that code for similar proteins in *T. carinatus* snake but have different expression site and divergent use.

Haemostasis & Toxins - Meeting Room 1&2 - 1430-1445

ABSTRACT NUMBER 19801 4CM3

COMPARATIVE STUDY OF HAEMOSTATIC FACTORS FROM THE VENOM GLAND OF THE COMMON BROWN SNAKE (PSEUDONAJA TEXTILIS) AND RELATED SPECIES.

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Snake venoms are an abundant source of proteins and other constituents that affect the haemostatic mechanism of mammals, including factor Xa-like prothrombin activators unique to Australian snake species. The aims of this study are to characterise those factors within the venom of the common brown snake (*Pseudonaja textilis*) that are responsible for alterations within the mammalian haemostatic mechanism. More specifically, the major objectives are two-fold. Firstly, to perform a comparative analysis of a factor Xa-like prothrombin activator gene from the venom of P. textilis and related species. Secondly, to develop a cDNA microarray from the venom gland of a coastal taipan (*Oxyuranus scutellatus*) for cross species comparisons via global expression patterns and identification of known and novel gene products that may have implications in the alteration of haemostasis. Application of this knowledge holds the potential for developing new diagnostic tools or novel haemostatic therapeutics, in particular, the development of a fibrin tissue sealant.

The full-length, cDNA transcript of the factor Xa-like prothrombin activator from the venom gland of six Australian snakes has been isolated and successfully cloned. Sequence alignments reveal a 72% degree of homology between the six proteases with a number of interesting structural properties, including high degrees of conservation surrounding cleavage sites. Attempts to express a recombinant form of the protease have commenced this year. Via the production of a cDNA library from the venom gland of the coastal taipan, a 4800 clone cDNA microarray chip was produced for the purposes of cross-species hybridisation and comparison of gene expression levels. Screening of this microarray is currently being performed, and has already identified a novel toxin related to that of a previously observed protein within the mulga snake (*Pseudechis australis*). It is hoped that through the analysis of global expression levels it may be possible to identify genes that may have implications for the coagulation mechanism of mammals, and thereby be of potential therapeutic or diagnostic benefit.

Haemostasis & Toxins - Meeting Room 1&2 - 1445-1500

ABSTRACT NUMBER 10701 4CM4

ISOLATION AND CHARACTERIZATION OF ANTICOAGULANT PROTEIN FROM THE VENOM OF HEMACHATUS HAEMACHATUS

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Snake venoms are complex mixtures of enzymatic and nonenzymatic proteins some of which exert profound effect on human hemostatic system. We have recently isolated and purified a non-enzymatic protein from the venom of *Hemachatus haemachatus* (South African ringhals cobra), using gel filtration and reverse phase high performance liquid chromatography, that exhibits anticoagulant activity. The protein has molecular mass of 6835.34 Da as determined by electron spray ionization mass spectrometry. This protein has been identified as a three-finger toxin from its amino acid sequence and it exhibits 80% homology to hemolytic protein 12B of *Hemachatus haemachatus*. The anticoagulant potency of this protein is comparable to the nonenzymatic anticoagulant cardiotoxin SXII from *Naja nigricollis* in the prothrombin time assays. We are interested in determining its structure-function relationship.

Haemostasis & Toxins - Meeting Room 1&2 - 1500-1515

ABSTRACT NUMBER 07402 4CM5

NEW BIOLOGICAL ACTIVITY OF HF3, AN HEMORRHAGIC METALLOPROTEINASE ISOLATED FROM BOTHROPS JARARACA VENOM, ON MACROPHAGE FUNCTION.

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HF3, a P-III class metalloproteinase isolated from the *B. jararaca* venom, is a potent hemorrhagic toxin, which degrades fibronectin, fibrinogen, fibrin, type I collagen and gelatin in vitro [1,2]. A cDNA encoding HF3 indicated that it is a multidomain molecule composed of a pro-domain, a catalytic domain with the characteristic zinc binding sequence, followed by disintegrin-like and cysteine-rich domains. It is known that metalloproteinases play a relevant role in the pathogenesis of viperine venom-induced local tissue damage including inflammatory reactions. In this study the effects of native HF3 and the recombinant disintegrin-like/cys-rich domains (DC/HF3) on C3b receptor-mediated phagocytosis of macrophages were evaluated. Native HF3 was isolated as described elsewhere [1] and DC/HF3 was expressed as a fusion protein with glutathione S-transferase (GST) in *E. coli* BL21 cells using the expression vector pGEX-4T1. The pure recombinant protein was obtained after cleavage with thrombin and was analyzed by N-terminal amino acid sequencing and Western blotting. Macrophages (mf) were obtained from Swiss mice peritoneal cavity 96 hours after intraperitoneal injection of thioglycolate (3%). Cell viability was measured by Trypan blue exclusion test. Phagocytosis via C3b receptor was studied with opsonized zymosan. All the studied proteins were non-cytotoxic for the elicited mf. Native HF3, GST-DC/HF3, DC/HF3 but not GST significantly increased the phagocytosis of opsonized particles by mf. The data shows the ability of venom P-III metalloproteinases to activate mf C3b receptors for phagocytosis and suggest that the disintegrin-like/cysteine-rich domains are important for this effect. Since phagocytosis is an innate and key event for host defense, P-III metalloproteinases and disintegrin-like/cysteine-rich domains may constitute relevant tools for studies on the physiology of effectors cells of immune responses.

[1] Assakura M.T., Reichl A.P., Mandelbaum F.R. (1986).). Toxicon. 24, 943-946.
[2] Mandelbaum, F.R., Assakura, M.T. (1988). Toxicon. 26, 379-385.

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Haemostasis & Toxins - Meeting Room 1&2 - 1515-1530

ABSTRACT NUMBER 21002 4CM6

THROMBOCYTE AGGREGATION CAUSED BY HEAD-TO-TAIL 3-ALKYLPYRIDINIUM POLYMERS ISOLATED FROM THE MARINE SPONGE RENIERA SARAI IS PARTLY BLOCKED BY THE COMBINATION OF ACETYL SALICIC ACID, CLOPIDOGREL AND ABCIXIMAB

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Water soluble polymeric 3-alkylpyridinium salts (poly-APS; MW 18900 and 5520 Da) were isolated from the marine sponge *Raniera sarai*. In vitro it strongly inhibited cholinesterase (AChE) from different species. The toxin had no direct effects on coagulation rate, but it possessed considerable haemolytic activity and induced the aggregation of small unilammellar lipid vesicles in vitro. Spectrophotometric measurements showed that the speed and intensity of platelets aggregation was dose dependent. Data obtained on blood samples from animals treated with poly-APS also revealed numerous thrombocyte aggregates. A quantitative assessment of the effects of poly-APS on platelets was performed by use of optical aggregometry. Washed platelets were incubated with the following final concentrations of poly-APS (mg/ml): 1.08, 0.1, 0.01, 0.001, 0.0001 and measured by means of optical aggregometry. Thrombocyte aggregation due to a different concentrations of poly-APS was modulated by addition of either acetylsalicylic acid (Aspegic), clopidogrel (Plavix) and abciximab (Reo-Pro) in 0.5 mg/ml, 0.5 mg/ml and 0.06 mg/ml concentrations, respectively or in combination of all the agents in the previously mentioned concentrations. At the highest concentration of poly-APS, Aspirin lovered the aggregation for 40 %, Plavix for 10% and ReoPro for 8%, and the combination of all three for 50%. The speed of aggregation is much slower when Aspirin is added than in case of Plavix or ReoPro. Our data suggest that activation of cyclooxygenase pathway is substantially important in the thrombocyte aggregation caused by Poly-APS. It seems that ADP-receptor activation and GP IIa/IIIb are important in the late phase of the Poly-APS induced thrombocyte aggregation.

Clinical Toxinology - Meeting Room 3 - 1400-1415

ABSTRACT NUMBER 04701 4CR1

CLINICAL TOXINOLOGY - WHERE TO NOW?

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Clinical toxinology is on the verge of recognition widely as a legitamate area of specialist expertise in medicine. For recognition to be effective, there must be an acceptable training and accreditation path. It is probably not viable to establish a separate specialist medical college just for clinical toxinology, so which existing college framework could it fit into? Currently the most interest is expressed from the emergency medicine field, but other possibilities exist and college structures and areas of interest vary from country to country. An alternative might be to form a clinical section within the IST, to administer accreditation globally, in cooperation with suitable colleges in each country. Accreditation will be dependant on adequate training. Only one international course currently exists, plus several other courses, either regional in focus or without a clinical focus. The needs of training, existing resources and strategies for the future will be discussed.

Clinical Toxinology - Meeting Room 3 - 1415-1430

ABSTRACT NUMBER 14202 4CR2

PROSPECTIVE STUDY OF DEFINITE CATERPILLAR EXPOSURES IN AUSTRALIA

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Exposure to caterpillars results in a variety of clinical effects depending on the species involved. The aim of this study was to describe the clinical effects from caterpillar exposures within Australia. Cases were recruited prospectively from calls to a poison information centre. Subjects were included if they had a definite exposure and they had collected the caterpillar or cocoon. The caterpillars were identified to genus and species level where possible. There were 36 included cases: 2 were contact exposures to caterpillar contents, 1 was an ingestion of a caterpillar and the remaining 33 patient had definite reactions from caterpillar or cocoon exposure. There were 5 families of caterpillars identified in the study: Artcidae, Limacodidae (*Doratifera* spp.), Anthelidae (*Chelepteryx* and *Anthela* spp.), Lymantriidae (*Euproctis edwardsis*) and Sphingidae, many of which occur worldwide. Clinical effects ranged from severe pain and wheal formation, to urticarial responses with erythema and papular rashes, depending on the species involved. There were no adverse effects following the caterpillar ingestion. Treatment consisted primarily of removal of the caterpillar or cocoon. Other treatment measures consisted of symptomatic treatment such as ice packs and antihistamines. This is the first prospective study of caterpillar exposures within Australia and demonstrates that exposures can result in a variety of reactions depending on the family and species involved.

Clinical Toxinology - Meeting Room 3 - 1430-1445

ABSTRACT NUMBER 22701 4CR3

EPIDEMIOLOGY OF SNAKEBITE IN THE MEKEO REGION OF CENTRAL PROVINCE, PAPUA NEW GUINEA

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Previous studies of snakebite in Papua New Guinea have identified the Mekeo region of Central Province as having one of the highest incidence rates for snakebite in the world (Lalloo et al, 1995). We have retrospectively examined 687 cases of suspected snakebite admitted to 3 major rural health centres (Veifa'a, Bereina and Inauaia) in the Mekeo region over the period January 1997 to December 2001.

Incidence, envenomation and mortality rates for snakebite in the region will be presented, along with detailed analysis of the clinical syndromes of envenoming. Data on the use of conventional and traditional methods of first aid are provided, along with antivenom usage, referral rates to tertiary care facilities, and the use of traditional/alternative remedies.

Presumptive determinations of the biting species are provided based on clinical presentation, and the appropriateness of current management practices, including antivenom selection and availability will be considered. A practical strategy for the management for real and suspected snakebites in rural, southern Papua New Guinea based is proposed.

Reference:

LALLOO DG, TREVETT AJ, SAWERI A, et al. The epidemiology of snakebite in Central Province and National Capital District, Papua New Guinea. Trans Roy Soc Trop Med Hyg. 1995 89:178-182

Clinical Toxinology - Meeting Room 3 - 1445-1500

ABSTRACT NUMBER 14201 4CR4

AUSTRALIAN MYGALOMORPH SPIDER BITE, INCLUDING FUNNEL WEB SPIDERS (ATRACINAE) AND MOUSE SPIDERS (MISSULENA SPP).

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A number of mygalomorph spider families cause bites in Australia, including the funnel web spiders (Atracinae: *Hadronyche* and *Atrax*) and mouse spiders (Actinopodidae:*Missulena*). There is ongoing debate about the significance of bites by mouse spiders and the frequency of severe envenoming by FWS bite. We conducted a prospective cohort study of definite spider bites with expert spider identification and include the analysis of mygalomorph spiders here. Subjects were recruited prospectively from February 1999 to April 2003 from patients presenting to participating hospitals or contacting a state Poison Information Centre. Forty nine cases of bites by mygalomorph spiders were included: 16 were by funnel web spiders, 13 by mouse spiders and 20 by other trapdoor spiders (Families Idiopidae and Nemesiidae). The local effects and circumstances of bites by mygalomorph spiders as a group were characteristic compared to other spiders. The following were highly statistically associated with mygalomorph spider bites ($p<0.0001$): circumstances (gardening at the time [Likelihood ratio (LR) 7.9] and distal limb bites [LR 2.0]) and early clinical features (presence of puncture marks OR bleeding [2.3] or severe pain [2.0]). Of the 16 FWS bites, there were ten cases with minor local effects, four with moderate envenoming (non-specific systemic or local neurotoxicity) and two with severe envenoming requiring antivenom. In addition to local effects, mouse spider bites caused local paraesthesia in three cases, local diaphoresis in one case and systemic effects (headache or nausea) in five cases, but not severe envenoming. Because these mygalomorph spiders appear similar to the non-expert, any bite by a large black spider should be treated as a FWS bite, and cases where the spider is not caught or seen, the above characteristic features can be used to identify patients that should be observed for a suspected FWS bite.

Clinical Toxinology - Meeting Room 3 - 1500-1515

ABSTRACT NUMBER 20301 4CR5

FIRST AID FOR POTENTIALLY LETHAL JELLYFISH STINGS IN AUSTRALIA

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The first aid management in Australia of jellyfish stings remains controversial, as there is limited evidence available. Recent data presented has resulted in the Australian Resuscitation Council changing some of its recommendations about first aid.

This paper will discuss beachside cardiopulmonary resuscitation, the role of vinegar, doubts raised about the use of pressure immobilisation bandages and the possiblity of heat inactivating jellyfish venom. *Chironex fleckeri* antivenom is currently carried by ambulances for use in the prehospital environment in Queensland, although we question the intramuscular route of administration, and whether there is any evidence that this antivenom has prevented a prehospital death.

We will present a suggestion for first aid for potentially life threatening jellyfish stings in Australia.

Clinical Toxinology - Meeting Room 3 - 1515-1530

ABSTRACT NUMBER 08001 4CR6

SNAKE VENOM NEUTRALIZATION BY INDIAN MEDICINAL PLANTS (VITEX NEGUNDO AND EMBLICA OFFICINALIS) ROOT EXTRACTS.

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Snakebite is a major health hazard that leads to high mortality rate especially in India. *Vipera russellii* and *Naja kaouthia* are the common snakes found throughout India and a large number of deaths occurr due to envenomation by these snakes. Antiserum is the only therapeutic agent available throughout the world. Antiserum sometimes does not provide enough protection against venom induced haemorrhage, necrosis, nephrotoxicity and often produced hypersensitive reactions. Antiserum development in animal is time consuming, expensive and requires ideal storage condition. Over the years many attempts have been made for the development of snake venom antagonists especially from plants sources. Many Indian medicinal plants are recommended for the treatment of snakebite but so far no systematic analysis has been done. A few preliminary reports exist which neither successfully confirmed their action nor active constituents. The present investigation explored the snake venom neutralizing activity of the plant extracts (*Vitex negundo* and *Emblica officinalis*) in rodents. The methanolic root extracts of *Vitex negundo* Linn. and *Emblica officinalis* Gaertn. were explored for the first time for antisnake venom activity. The plant (*V. negundo* and *E. officinalis*) extracts significantly antagonized the *Vipera russellii* and *Naja kaouthia* venom induced lethal activity both in in vitro and in vivo studies. *Vipera russellii* venom induced haemorrhage, coagulant, defibrinogenating and inflammatory activity was significantly neutralized by both plant extracts. No precipitating bands were observed between the plant extract and snake venom. The above observations confirmed that the plant extracts possesse potent snake venom neutralizing capacity and need further investigation.

Toxins as Tools - Hall C - 1600-1615

ABSTRACT NUMBER 11801 4DH1

CLONING, EXPRESSION AND CHARACTERIZATION OF A BI-FUNCTIONAL DISINTEGRIN/ ALKALINE PHOSPHATASE HYBRID PROTEIN

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Integrins are transmembrane heterodimeric glycoproteins responsible for cellular communication; therefore they play an essential role in many physiological events. Viper snake venoms contain integrin antagonists called disintegrins which bind and inhibit integrin function. They present a loop containing an RGD motif responsible for integrin binding. The engineering of disintegrins fused to a reporter enzyme will be an interesting approach to build integrin markers. Even more, the disintegrin scaffold could be used to present other protein binding motifs. In this work we have obtained alkaline phosphatase (APv) tagged eristostatin (Er) by cloning and expressing eristostatin synthetic gene into the pLIP6-GN vector. Eristostatin, a 49-residue disintegrin binds selectively to αIIbβ3 integrin, blocking its binding to fibrinogen and consequently inhibiting ADP-induced platelet aggregation. The resulting fusion protein, Er/APv, was identified by SDS-PAGE and by western blotting using both anti-Er and anti-AP antibodies. This fusion protein showed enzymatic AP activity similar to wild APv and its potential use for an αIIbβ3 integrin assay was tested in a one-step dot blot using immobilized cells incubated with the marker and developed by AP substrate. Er/APv showed selectivity towards platelets and αIIbβ3 integrin transfected cells and reacted with the same region as unlabeled Er, as analyzed in competition assays. Our data present a novel tool, Er/APv, with potential use as molecular marker in processes where the αIIbβ3 integrin is involved.

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Toxins as Tools - Hall C - 1615-1630

ABSTRACT NUMBER 14501 4DH2

GLYCEROTOXIN FROM GLYCERA CONVOLUTA STIMULATES NEUROSECRETION BY UP-REGULATING N-TYPE CA2+ CHANNEL ACTIVITY

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Glycerotoxin from the venom of *Glycera convoluta*, is a novel 320 kDa protein capable of reversibly stimulating spontaneous and evoked neurotransmitter release at the frog neuromuscular junction. By sequential and selective inhibition of various types of Ca^{2+} channels, we found that Glycerotoxin was acting via Cav2.2 (N-type). In neuroendocrine cells, it elicits a robust albeit transient influx of Ca^{2+} sensitive to Cav2.2 blockers ω-conotoxin GVIA and MVIIA. Moreover, Glycerotoxin triggers a Ca^{2+} transient in HEK cells overexpressing Cav2.2 but not Cav2.1 (P/Q-type). Whole-cell patch-clamp analysis of Cav2.2 expressing HEK cells revealed an up-regulation of Ca^{2+} currents due to a leftward shift of the activation peak upon Glycerotoxin addition. A direct interaction between Cav2.2 and this neurotoxin was revealed by co-immunoprecipitation experiments. Glycerotoxin is therefore a unique addition to the arsenal of tools available to unravel the mechanism controlling Ca^{2+}-regulated exocytosis via the specific up-regulation of Cav2.2.

Toxins as Tools - Hall C - 1630-1645

ABSTRACT NUMBER 20801 4DH3

ACHE - INHIBITORY PSEUDOZOANTHOXANTIN-LIKE COMPOUND FROM PARAZOANTHUS AXINELLAE ADRIATICUS IS PARAMAGNETIC - A NEW AND SPECIFIC CONTRAST AGENT FOR THE MAGNETIC RESONANCE IMAGING?

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Parazoanthus axinellae is a common species inhabiting underwater caves and crevices of Mediterranean sea. In a search for new biologically active compounds from marine organisms we found that a pseudozanthoxantin isolated from *P. axinellae adriaticus* binds to acetylcholinesterase as a reversible inhibitor, similar to the action of physostigmin. Here we report some paramagnetic characteristics of the new AChE inhibitor. The specimens of *Parazoanthus axinellae adriaticus* (Pax) were collected by SCUBA diving in the waters of island Cres (Adriatic sea, Croatia). They were frozen and transferred to the laboratory, where we kept them at -20^0 C until use. Homogenate of the organism (250 ml) was extracted with 750 ml of absolute ethanol. A portion of the dark orange supernatant (1 ml) was passed through the Millex-GV 22 μm filter unit (Millipore, USA) and used for further purification by semi-preparative HPLC column. Active fractions were pooled again and dried as described before. Purity of rechromatographed samples was checked on the precoated F254 silicagel plates (Merck, FRG) using chloroform : methanol : 25% NH4OH in a 80 : 20 : 2 ratio (v/v) as a solvent. The isolation procedure gave analytically pure compound with a molecular mass of 242 Da determined by EI-solid probe mass spectroscopy. H-NMR spectra revealed the structure of the isolated compound which is probably identical to pseudozoantoxanthin H-NMR was measured in deuterated methanol or deuterated DMSO using XL 300 MHz Varian instrument (Varian). AChE inhibitory activity was measured on purified electric eel AChE and on human erythrocyte-bound AChE, on human plasma butyrilcholinesterase (BuChE) and on the bovine cervical ganglion AChE. Inhibitory activity was expressed in inhibitory units. 1 inhibitory unit was defined as the amount of inhibitor (calculated from the dry weight of preparation) which inhibits the AChE activity by 50% at 25^0 C. The substance was dissolved in water in 1, 10, 100 nM and 1 μM concentration. T1 and T2 decay times were measured on a 2,25 T Bruker tomograph, and data were compared to T1 and T2 of water and control substances. The results show that T1 remained nearly unchanged (10 ±4%), while T2 increased 40 ±4% with 10 nM or higher concentration of Pax. As the substance is reasonably lipophilic it crosses the blood-brain barrier and might be used for identification of AChE reach regions of brain, provided that the relative concentration reaches 10 nM. Modified substances with high affinity for AChE but even weaker AChE inhibitory activity may be developed as relatively safe contrast agents that could be used in neurobiological studies.

Toxins as Tools - Hall C - 1645-1700

ABSTRACT NUMBER 11101 4DH4

NEUROTOXIC PEPTIDE TRANSLOCATION THROUGH AN INSECT GUT.

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Due to the progress in the fields of molecular biology and biotechnology the clonable polypeptides gain increasing importance as pharmaceutical and pesticidal agents. Administration via the oral route requires that the agent has sufficient solubility, sufficient stability in the intestinal lumen and possesses the ability to cross the intestinal wall. Those considerations have motivated and directed our studies on the penetrability of venom derived neurotoxic and insect selective polypeptides through an insect gut. In our previous studies certain toxic polypeptides (~7-8 kDa) such as the amphipathic cobra venom cytotoxin [1,2] and the polar insect selective AaIT neurotoxin [3,4] were shown by the aid of microscopical autoradiography to cross the midgut of Sarcophaga flies. Occurred in a progressive frontal / "diffusional" fashion throughout the cytoplasm and entire length of the columnar gut epithelial cells. It has been shown that the polypeptides readily pass through the peritrophic membrane, penetrate the epithelial gut cells from the apical region through the microvilli and cross the entire gut wall within 30-60 min. [5,6,7]. That about 0.3 % of the ingested AaIT toxin have crossed the gut in an intact non degradated form and oral toxicity was about 700 times lower than its injection toxicity [7]. The above data have demonstrated that the digestive system of Sarcophaga flies is permeable to proteins and polypeptides.

Recent assays on oral delivery of neurotoxic polypeptides to Spodoptera moths have revealed that:

1. Crude scorpion venoms induce paralysis within 10-30 min.
2. Isolated fractions or pure polypeptides induce paralysis within 1-3 min.
3. The orally induced paralysis is enhanced by proteolytic inhibitors.

It is suggested, therefore that the orally induced paralysis of the moth is a consequence of a fast gut crossing by a neurotoxic polypeptide which affects excitable target tissues in the body. We assume that gut translocation in affected by the "leaky" gut morphology, its reduced proteolytic and denaturating capacity and the presence of attachment / internalization sites on the epithelial apical membranes.

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Clinical Toxinology - Meeting Room 3 - 1600-1615

ABSTRACT NUMBER 02303 4DR1

THE CARDIOVASCULAR ACTIONS OF THE IRUKANDJI (CARUKIA BARNESI) JELLYFISH

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The cardiovascular activity of crude venom extracted (CVE) from the tiny *Carukia barnesi* (*C. barnesi*) jellyfish, a known cause of the Irukandji syndrome, was assessed. The *C. barnesi* CVE (0.1-10μg/ml) caused a tachycardia in the guinea pig isolated and rat right isolated atria in the presence of atropine (1μM), and a bradycardia when tested in the presence of propranolol (1μM). In the paced rat and guinea pig left atrium, CVE (0.1-3μg/ml) elicited a positive inotropic effect in the presence of atropine. Pretreatment of the rat atria with either ω-conotoxin GVIA (0.1μM) or tetrodotoxin (0.1μM) abolished CVE activity. The CVE (0.1-30μg/ml) contracted the rat mesenteric small artery. Pretreatment with tetrodotoxin, ω-conotoxin GVIA or prazosin (0.3μM) did not significantly alter the arterial contraction to CVE. Box jellyfish antivenom (92.6 Units/ml) neutralised the vasoconstrictor effects of *Chironex fleckeri* (*C. fleckeri*) venom in the rat mesenteric artery but did not alter the effects of *C. barnesi* venom in the rat artery or right atria preparations. The CVE (10 μg/ml) caused a biphasic response in the human isolated right atrial trabeculae causing a rapid reduction in the contractile force [minimal effect] followed by a more sustained increase in contractile force [maximal effect]. In the presence of propranolol (200nM), the minimal effect was persistent but the maximal effect was markedly reduced. The converse was true when the trabeculae contractility was tested in the presence of atropine. CVE (180μl/kg) infused intravenously in anaesthetised small pigs caused tachycardia and systemic and pulmonary hypertension. Peripheral venous blood samples demonstrated a marked elevation in circulating noradrenaline and adrenaline, at the peak of the tachycardia and systemic hypertension. We conclude that *C. barnesi* venom contains a sodium channel modulator that stimulates the release of excessive catecholamines explaining, at least in part, the clinical features of the potentially deadly Irukandji syndrome.

Clinical Toxinology - Meeting Room 3 - 1615-1645

ABSTRACT NUMBER 20302 4DR2-3

IRUKANDJI SYNDROME: MORE THAN ONE JELLYFISH

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Barnes in 1961 demonstrated that a small jellyfish *Carukia barnesi* was responsible for causing the Irukandji syndrome. After capturing this jellyfish he stung himself, the on duty lifeguard and his 9 yo son. Within 30 minutes all developed significant distress, with severe abdominal, chest, limb and back pain, nausea & vomiting, classical symptoms of Irukandji syndrome.

Since this experiment, *Carukia barnesi* has been known as the Irukandji jellyfish. Other jellyfish have been suspected as causing Irukandji syndrome however no evidence has been provided.

Today a clinican and a biologist will present a series of cases of Irukandji syndrome over the last seven years in Cairns and demonstrate that these cases were due to non *Carukia barnesi* jellyfish.

This is the first time that non *Carukia barnesi* jellyfish has been identified and has significance for the epidemiology of the condition, and for researchers contemplating the production of an Irukandji syndrome antivenom.

Clinical Toxinology - Meeting Room 3 - 1645-1700

ABSTRACT NUMBER 12401 4DR4

IN VIVO EXAMINATION OF TREATMENT STRATEGIES FOR BOX JELLYFISH (CHIRONEX FLECKERI) ENVENOMING: EFFICACY OF BOX JELLYFISH ANTIVENOM (CSL) AND MAGNESIUM SULPHATE

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The box jellyfish (*Chironex fleckeri*) has caused more than 65 deaths in the last century. This may be due to cardiac toxicity. Difficulties in obtaining pure venom samples have hindered the biochemical and pharmacological characterization of jellyfish venoms. However, a superior technique for the extraction of venom from the nematocysts has been developed. The aim of this study was to assess the efficacy of CSL box jellyfish antivenom and magnesium sulphate against the in vivo cardiovascular effects of *C. fleckeri* venom in rats. Magnesium sulphate has recently been suggested as a novel therapy against box jellyfish envenoming. Male Sprague-Dawley rats (280-350g) were anaesthetised with pentobarbitone sodium (60-100mg/kg; i.p., supplemented as required). A midline incision was made in the cervical region and cannulae inserted into the trachea, jugular veins and carotid artery. Venom (30 micro g /kg; i.v.) produced a transient increase in mean arterial pressure (MAP; 32.4 ± 2.3mmHg) followed by a rapid decrease in blood pressure and death in all animals (time to death = 220s ± 21s; n=10). Prophylactic administration of antivenom (AV; 3000U/kg) prevented death in 40% of animals (ie. 4 out of 10). Rats that died (60%; time to death = 167s ± 23s) displayed a similar transient hypertensive response (increase in MAP = 36.0 ± 3.4 mmHg) followed by a decrease in blood pressure, as seen in animals administered venom (30 micro g/kg) alone. Infusion of magnesium sulphate (0.05-0.07M; 0.25ml/min) for 20 min prior to the administration of venom (30 micro g/kg) did not prevent the venom-induced increase in MAP (45.9 ± 2.3 mmHg) nor death of animals (time to death = 266s ± 34s; n=13). However, the prophylactic treatment of animals with both AV and magnesium sulphate prevented death in 100% of animals (n=10). The results of this study suggest that magnesium sulphate, when used in conjunction with AV, can prevent *C. fleckeri* venom (30 micro g/kg)-induced death in rats. The toxin(s) responsible for the cardiotoxic effects of *C. fleckeri* venom, and the mechanism(s) involved in the protective action of magnesium sulphate require further elucidation.

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 21001 4PF.3

EQUINATOXIN II DECREASES INTRACELLULAR PH IN ENDOTHELIAL CELL LINE ECV-304

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Equinatoxin II is a pore-forming actinoporin. It causes vasoconstriction only in the presence of intact endothelium. Therefore its effects on endothelial cells. Endothelial cell line ECV-304 was used in all the experiments. Cells (80-100 cells per plate) cultured in standard culture media (MEM), were transferred to Krebs-Henseleit (K-H) solution before the experiments, and then treated by one of the following EqT II concentrations (nM): 0.5, 1, 10, 100. Morphologic changes of the cells were observed by means of the laser scanning confocal microscopy (Leica, Germany). In the other group of experiments K-H solution was substituted by K-H without calcium, or 1 μM nicardipine was added to usual K-H solution. In one group of the experiments K-H solution was substituted with 320 mM isotonic saharose. Intracellular pH was measured microspectrofluorometrically using Fura-2 SNARF®-1 (both as acetoxymethyl ester, acetate). At 1 nM and 10 nM concentrations of EqT II the diameter of the cells rose to 150-250 % of the control values within the first five minutes. 20 min later all the cells were lysed. At 100 nM all the cells were lysed within the first three minutes after the addition of the toxin. Less than 5% of the cells were lysed after addition of 100 nM of the toxin in case of K-H without calcium in the medium. Addition of calcium ions to those cells caused the lysis of the cells a dose dependent manner (see above). Nicardipine did not affect the EqT II induced changes in cell morphology. In isotonic saharose only 1.8 ±0.1 % of cells were lysed after the addition of 100 nM of the toxin. The inhibition of the toxin action by saharose may be explained by prevention of osmotic shock and consequent lytic action. Intracellular pH dropped to 6,1±0.2 units only when Ca was present in medium. The fall of intracellular pH was faster at higher doses of EqT II. The results support the conclusion that calcium ions are the intracellular messengers of the effects of equinatoxin II on the isolated endothelial cells. Increase of intracellular calcium concentration and the drop of intracellular pH may also play a role in the cytolytic action of the toxin.

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 08101 4PF.3

TETRODOTOXINS OF THE STARFISH ASTROPECTEN VAPPA COLLECTED FROM WESTERN TAIWAN

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The specimens of the starfish *Astropecten vappa* were seasonally collected from Chiayi Prefecture, western Taiwan and assayed for toxicity from April to December 2001. The highest toxicity of total specimens was observed for specimens collected in December with a value of 827 mouse unit(MU)/specimen by using tetrodotoxin(TTX) bioassay. The highest value of seasonally average toxicity in viscera and other parts were 30°±18 and 12°±6 MU/g, respectively, in December 2001. The toxin was partially purified by Diaflo YM-1 membrane ultrafiltration and Bio-Gel P-2 column chromatography. HPLC and GC-MS analyses demonstrated that the starfish toxin consisted of TTX and anhydrotetrodotoxin(anh-TTX). Furthermore, the starfish *A. vappa* is first reported to contained TTX.

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 12201 4PF.3

ENGINEERING OF A CONUS TOXIN INTO A POTENT αIIBβ3 INHIBITOR

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α-conotoxins are neuronal-specific toxin isolated from the venom of cone shells. These cysteine-rich peptides are conformationally constrained by two disulfide bridges, making them useful as exceptional candidates for peptide scaffolds. In this project, we have included a RGD tripeptide sequence which plays a crucial role in adhesive reactions into the mini protein scaffold. Insertion of the short RGD segment was performed on both the 1st, and the 2nd intercysteine loop of the conotoxin scaffold. The two hybrid sequences were synthesized by solid-phase peptide synthesis methods and were purified by chromatography. Folding of the engineered conotoxin revealed 3 isoforms in each of the 2 sequences. Preliminary in vitro experiments revealed that the anti-platelet effect of the RGD segment was retained in the engineered peptide, with the isoform of highest potency having an IC50 of 0.22 μM when RGD was inserted into intercysteine loop 1, and the IC50 of 2.7μM for the most potent isoform with the tripeptide inserted into the 2nd intercysteine loop. These results show that the compact conotoxin scaffold may prove to be invaluable for the development of potent, orally active biopeptides.

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 12301 4PF.3

DETERMINATION OF ALPHA-CONOTOXINS BINDING MODES ON NEURONAL NICOTINIC RECEPTORS.

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The venom peptides (conotoxins or conopeptides) evolved by cone snails for prey capture and defence are small, highly structured mini-proteins with diverse pharmacology. The X-ray and NMR structures for most classes of conotoxins have now been determined. We describe here the construction of a homology model for the neuronal alpha7 and alpha3beta2 nicotinic acetylcholine receptors based on the acetylcholine binding protein (AChBP) structure and the use of the GOLD docking program to identify the binding sites for four alpha-conotoxins: ImI, PnIA, PnIB and MII. Using this structural approach, we identified on these models 2 cavities around the putative acetylcholine binding site: a small one above the b9/b10 antiparallel loop, and a larger one below it. The docking results suggest that the alpha-conotoxins bind to receptor residues located mainly in and above the acetylcholine binding site, with part of their structure buried in the small cavity. This contrasts with the alpha-neurotoxins that enter the binding site from the larger cavity below the beta hairpin. Residues in these cavities seem to be responsible, at least in part, for the toxin selectivities. Validation of these models of α-conotoxins/nAChRs interactions will be carry out by mutant cycle analysis. The structural restraints derived from mutagenesis studies will be used to refine models of these membrane proteins. This should define a more accurate pharmacophore for these important therapeutic targets that could lead the design of new drugs.

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 12801 4PF.3

PEPTIDE INHIBITORS OF MYONECROTIC [LYS49] PHOSPHOLIPASES A_2 DERIVED FROM ENZYMATIC DIGESTION OF SNAKE SERUM PROTEINS

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Phospholipase A_2 (PLA_2) (EC 3.1.1.4) isozymes, which are the major toxic components in snake venoms, catalyze the hydrolysis of the 2-acyl ester bond of 3-sn-phosphoglycerides. Snake venom PLA_2 are known to exhibit a variety of physiological activities such as hemolysis, edema-inducing, neurotoxity and myotoxicity. Potent peptide inhibitors for PLA_2s are useful for analyzing diversified physiological functions of PLA_2s and may be available for therapeutics of snakebite or inflammatory diseases. Many venomous snakes are resistant to their own venoms due to the presence of neutralizing proteins in their sera. Peptide fragments of the proteinous inhibitors containing an active inhibitory domain may also act as inhibitor.

In the present study, we developed novel peptide inhibitors derived from enzymatic digestion of *Trimeresurus flavoviridis* (habu) serum proteins. To construct peptide libraries containing inhibitors, habu serum proteins were digested by various proteases with different specificities such as pepsin, a-chymotrypsin, elastase, protease A and actinase E. The cleavages of serum proteins were monitored by SDS-PAGE and gel filtration chromatography, and their inhibitory activities against myonecrotic [Lys49]PLA2 (BPII) were assayed by using BHK-21 cells. Among the proteases employed, only actinase E gave the potent peptide fractions, which inhibited myonecrotic activity of BPII more effectively than habu serum itself. In this study, we purified and characterized the effective peptides by ultracentrifugation and gel filtration chromatography.

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 14102 4PF.3

TOXINS FROM THE VENOM OF THE SPIDER MACROTHELE GIGAS THAT BIND TO THE SITE-3 OF THE SODIUM CHANNEL

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Peptide toxins from spiders have proved to be useful agents for discriminating between different components of native ion channel currents, and for the molecular isolation and designation of cellular receptors. Five peptide toxins (Magi 1-5) were isolated from the Hexathelidae spider *Macrothele gigas*. The amino acid sequences of Magi 1, 2, and 5 have low similarities to the amino acid sequences of known spider toxins. The primary structure of Magi 3 is similar to that structure of the palmitoylated peptide named PlTx-II from the North American spider *Plectreurys tristis* (Plectreuridae). Moreover, the amino acid sequence of Magi 4, which was revealed by cloning of its cDNA, displays similarities to the Na^+ channel modifier δ-atracotoxins from the Australian spiders *Atrax robustus* (Hexathelidae). Competitive binding assays using several 125I-labelled scorpion toxins clearly demonstrated the specific binding affinity of Magi 1-5 to the site-3 of the insect sodium channel.

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 14301 4PF.3

INFLAMMATORY PATHOGENESIS OF SNAKE VENOM METALLOPROTEINASE-INDUCED SKIN NECROSIS

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Local tissue damage, characterised by oedema, haemorrhage and necrosis, is a common consequence of envenoming by many vipers. We have investigated the contribution of inflammatory responses induced by the venom metalloproteinase jararhagin (isolated from *Bothrops jararaca* venom) in the development of these lesions. Local venom effects (oedema, haemorrhage and necrosis) were induced experimentally in knockout mice deficient in the TNF receptors TNFR1 or TNFR2, IL-1_R, IL-6 and iNOS. Jararhagin-induced dermal necrosis was abolished in mice deficient in the TNF receptors TNFR1 and TNFR2, and the same activity was significantly reduced in IL-6-/- mice. There was no significant difference in oedema and haLemorrhage activities following jararhagin insult between knockout and wild-type (WT) strains, indicating that these local venom metalloproteinase-induced effects are independent of these pro-inflammatory mediators. The contribution of both TNF receptors and IL-6 in local tissue necrosis raises important therapeutic issues regarding the treatment of local envenoming.

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 15402 4PF.3

INDIVIDUAL VARIABILITY IN TITYUS SERRULATUS (SCORPIONES, BUTHIDAE) VENOM ELICITED BY MALDI-TOF MASS SPECTROMETRY

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Venom variability in specimens of *Tityus serrulatus* scorpion was assessed by mass spectrometry (MALDI-TOFMS) analyses. An expanded time lag venom extraction protocol was carried out using ten scorpions to study individual variations that might occur due to different rates in protein expression and/or processing. The first extraction of venom was made from the animals after 20 days of starvation, which allowed the venom gland to be filled up. The second extraction event was carried out 24 hours after the first one. The third was 8 days after the first extraction. By means of MALDI-TOF analyses, important variations were observed in venoms of a single specimen extracted at different times, specially in latter extraction events. These variations are most probably related to dynamics in cell gland production. Since *T. serrulatus* is a parthenogenetic species, sexual variations are naturally excluded and we did not expect intra-specific variations, which was confirmed. Knowledge of individual venom variability is extremely important to avoid misunderstandings in the use of venom proteomic analysis as a taxonomic tool.

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 16002 4PF.3

ANCIENT SNAKES WITH MODERN FANGS, OR VICE VERSA? A COMPARATIVE ELECTRON MICROSCOPIC STUDY OF VENOMOUS SNAKE FANGS FROM THE EARLY MIOCENE OF THE MAINZ BASIN, GERMANY.

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Vipers and pitvipers (family Viperidae) were long believed to be the most modern or advanced among living snakes because they possess the most effective venom apparatus of all snakes. This consists of large mucous-serous venom glands with considerable lumen, extensive associated muscles allowing for well-regulated high-pressure ejection of venom, and long tubular fangs which facilitate deep injection of the venom into prey or enemies. However, fossil remains resembling the vertebrae of modern viperid snakes were found at several sites in Eurasia and North America, suggesting that such organisms were widely distributed with the beginning of the Early Miocene (23 million years before present), and had possibly evolved much earlier. Moreover, recent molecular studies on the higher-level phylogeny of snakes consistently place viperid snakes basal to the remaining colubroid snakes, suggesting a scenario in which the members of the superfamily Colubroidea (which comprises the vast majority of living snakes) evolved from venomous ancestors, and in the course of their evolutionary diversification several times experienced a reduction or loss of their venom apparatus. An unknown variable in this theory has been the state of the venom-delivery system of the earliest known vipers. We used scanning electron microscopy to study a series of snake fangs from the Oppenheim/Nierstein Quarry (Early Miocene, Mainz Basin, Germany) and compared them to the fangs of various genera of extant viperid and elapid snakes. We identified two distinct fang morphs among the fossil material: The majority type, a tubular fang, was virtually identical in details such as fang length and curvature, length and shape of venom discharge orifice, and prominent ridges, to the fang of a modern European viper (Vipera ammodytes). The second fang type was grooved and comparable to the fangs of certain elapid snakes. Our findings suggest that in viperid snakes, the fangs may have undergone only minimal, if any, changes in the past 23 million years. It is likely that the evolutionary processes shaping this highly effective venom-delivery system date back earlier, and that a much longer period must be postulated for the evolutionary history of snake venom toxins.

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 16103 4PF.3

SPATIAL STRUCTURE AND CONFORMATIONAL HETEROGENEITY OF A WEAK TOXIN FROM THE COBRA NAJA KAOUTHIA VENOM.

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The weak toxins from Elapidae venom belong to the superfamily of "three-finger toxins". There were no detailed spatial structures of weak toxins and their pharmacology is largely unknown. Contrary to other types of "three-finger toxins", weak toxins contain a unique disulfide bridge in the first loop. The detailed analysis of the spatial structure of weak toxin WTX from the venom of Naja kaouthia has been performed on the basis of 2D NMR spectroscopy data. For some residues of the first and second "fingers" of WTX the presence of two sets of resonances of approximately equal intensities was found, which is explained by the conformational heterogeneity of polypeptide owing to 'cis-trans' isomerisation of the Arg32-Pro33 peptide bond. The data obtained (NOE, spin-spin coupling constants and rates of amide protons exchange to deuterium of the solvent) allowed us to determine spatial structure of WTX.

Both 'cis' and 'trans' isomers adopt an overall structure similar to other "three-finger toxins" formed by two antiparallel beta-sheets. First of them consist of two-strands (regions 1-5 and 13-17) linked by loop made up of 7 residues including disulfide bridge 6-11. The second, three-stranded, beta-sheet consists of two strands from central "finger" (regions 23-28 and 38-43) and one strand from third "finger" (residues 55-59). The relatively mobile tip of central "finger" (region 29-37) possess two forms corresponded to the 'cis' or 'trans' conformation of the Arg32-Pro33 peptide bond. C-terminus of the WTX is close in space to the N-terminal beta-strand.

Obtained spatial structure of the WTX is compared with the known 3D structures of other "three-finger proteins" including Bucandin (*Bungaris candidus*) and cell surface protein CD59, which have similar disulfide bridge scaffold like WTX, Cytotoxin II (*N oxiana*), which also possess 'cis-trans' isomerisation of the peptide bond, as well as with Neurotoxin II (*N oxiana*) and alpha-Cobratoxin (*N siamensis*), which have the typical 3D structures of short and long alpha-neurotoxins, correspondingly.

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 16501 4PF.3

FIVE DISULPHIDE BRIDGED THREE-FINGER TOXINS FROM NAJA SPUTATRIX

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cDNA encoding a long-chain neurotoxin has been cloned and sequenced from the venom gland of Malayan spitting cobra, *Naja sputatrix*. This long-chain neurotoxin exhibits 88% sequence similarity and about the same level of toxicity as the long-chain alpha-neurotoxin from *Naja kaouthia*, alpha-cobratoxin. The structure and organization of the genes encoding the long-chain neurotoxins and four other isoforms of weak neurotoxins in the venom of *Naja sputatrix* are presented. All the genes contained three exons interrupted by two introns, a structure similar to other three-finger toxin family. Phylogenetic analysis of these genes showed that the weak neurotoxin gene confines to a distinct group. We observe that specific mutations of the genes provide the diversity in function in these toxins while maintaining a common structural scaffold.

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 16801 4PF.3

ACUTOBIN —A NEW ANTITHROBOTIC AGENT PURIFIED FROM DEINAGKISTRODON ACUTUS VENOM

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A thrombin-like enzyme was separated and purified from the venom of *Deinagkistrodon acutus* by chromatography and gel filtration with molecular weight about 26000 Da designated as acutobin. Pharmacological studies showed that it prevented thrombosis in rabbits and rats experimental models and possessed thrombolytic activities in rat and rabbit venous thrombosis models and rabbit arterial thrombosis models without direct fibrinolytic activitiy in vitro. The thrombolytic effect was caused by t-PA release from endothelium cell induced by acutobin as confirmed by in vitro and in vivo experiments. Furthermore,in magnetic resonance imaging studies on hyperglycemic rat middle cerebral ischemic/reperfusion model,,acutobin can reduce the extent of brain injury, significantly reduce the hemispheric diffusion weight image-detected lesion ,increased the survival time of rats. The ELISA technique was adopted for investigaing the pharmacokinetics of acutobin in rabbits. Three doses (0.5,1.0 and 2.0 u/kg) were iv bolus into the animals.The plasma concentration-time course of acutobin was described as three compartments model, the half life of the a phase was about 3.9~5.1 min, that of the ß phase about 42~60 min and γ phase about 18~19 hr. Threre were no significant difference in the parameters among the three phases in different doses except that of the central compartment that showed volume distribution were significant difference The pharmacokinetic studies in 14 healthy volunteers divided into three groups received iv infusion 1, 2 and 3 U of acutobin respectively, the blood concentration -time curve behaved as a open two-compartments model. The t1/2 α phase of the three doses were 0.66±0.1, 0.97±0.34, and 1.22±0.77 h and t1/2ß 15.95±2.40, 18.80±4.94 and 19.73±4.28 h respectively. Toxicity: .The LD50 iv in mice was 101.5(84.4-122.3) u/kg. Long term toxicity was performed in 12 dogs divided into 3 groups received iv injection 0.5 and 1.5 u/kg acutobin and normal saline respectivly every day consecutively for 28 days. There were no significant change in blood picture, functions of liver and kidney. Clinical trial: Phase 1. 18 health volunteers divided into 3 group received in 1,2 and 3 U acutobin respectively. The results of 3 groups showed that the enzyme inhibited platelet aggregation, decreased platelet count, prolonged partial thromboplastin time , lowered fibrinogen level, increased plasma fibrin degraded product, shortened euglobulin lysis time with dose-response relationship. The plasma ALT,AST,ALP, total protein, albumin, globulin,total, cholesterol, bilirubin, urea nitrogen, creatineine,WBC and hemoglobin were in normal range. These results suggested that the enzyme in dose as high as 3 units caused no adverse reaction on liver. kidney and blood. The respiration, blood pressure ,pulse rate and temperature also no significant change. Some scattered petechiae appeared around the culf of the blood pressometer, but disappeared spontaneously after 2 hours in 2 cases of high dose group. Phase 2: To evaluate the safety and efficacy, the acutobin was tested in patients with acute ischemic stroke administered within 7 days of stroke onset in a double-blind, randomized, placebo-centrolled trial. 100 patients received intravenous infusion of acutobin 0.75U and 100 patients received placebo daily for 7 days and another 7 day after 5 days cessation interrupt. Neurological outcome, disability, platelet aggregation and some laboratory parameters were measured. The results showed that, there was a significant improved ($P<0.05$) neurological outcome on the Yangzhou (modified Eidenberg and Scandinavian) Stroke scale. The platelet aggregation rate, plasma viscosity, fibrinogen level, hematocrit and thrombin time were decreased. The total efficacy rate (included cured and improvement) was 92.2%. No bleeding occurred except 4 patients had minor symptomatic hemorrhagic infarction in MRI, but recovered spontaneously without any special treament. In 200 cases of open trial, a similar result was obtained . Phase 3. 1341 patients with acute ischemic stroke within 7 days of stroke onset were enrolled in multicenter included 40 hospitals participants in China for open trial according the program as phase 2. The results were similar as phase 2 with total efficacy rate 94.2%. Conclusion: Acutobin appears safe and effective for acute ischemic stroke when administered in patient within 7 days of onset of the disease

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 17601 4PF.3

IDENTIFICATION OF SMALL TOXIN GENES FROM THE VENOM GLAND OF OPHIOPHAGUS HANNAH USING ESTS APPROACH

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Snake venoms are complex mixtures of bioactive compounds, including enzymatic and non-enzymatic proteins, as well as low molecular weight components (metals, peptides, lipids, nucleotides, carbohydrates and amines). In an attempt to search for novel proteins from *Ophiophagus hannah* (king cobra) venom, we generated ESTs of the most abundantly expressed mRNAs from *Ophiophagus hannah* (king cobra) venom gland. At first, RACE library of the venom gland of King cobra was made. Minimal cycles of PCR were then performed to enrich the gene copy numbers before cloning and sequencing. We randomly selected 242 clones for sequencing. Our results indicate that 216 clones have inserts ranging from 100 to 700 bp. Nucleotide sequences of a large number of these clones (154; 65%) showed homology to *Pseudonaja textilis* long neurotoxin precursor (PT-LNTX, accession no: AF082982) and its variants. Clone 197 shares 66% identity and 74% homology with a cytotoxin homolog S3C2 (CXH2 ASPSC) and clone 141 shares 55% identity and 71% homology with the weak toxin DE-1 (TXW1 OPHHA). Thus these two clones appear to encode new three finger toxins and were selected for further characterization. Using this simple and rapid approach, we managed to identify small genes from king cobra venom gland.

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 18002 4PF.3

VENOM OF THE BROWN TREESNAKE (BOIGA IRREGULARIS): A MODEL FOR COLUBRID VENOM ANALYSIS

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Venoms of snakes of the polyphyletic family Colubridae offer an immense opportunity for the discovery of novel biological compounds and new structural variants, and few colubrid species have been thoroughly studied. One of these, the brown treesnake (*Boiga irregularis*), is an invasive generalist predator which has caused an ecological disaster on the small island of Guam. We initiated a study of brown treesnake venom composition with the general goal of identifying all dominant protein species, a feasible goal because these venoms are of relatively low complexity. We chose this snake species because it has been implicated in numerous human envenomations, it is readily obtained in large numbers, it is large (up to 2.5 meters) and it produces a large amount of venom, facilitating analysis and purification. Using ketamine-HCl and pilocarpine-HCl, yields of up to 25 mg per extraction have been obtained from large individuals. Venoms were analyzed using enzyme assays, one and two dimensional electrophoresis, HPLC, MALDI-TOF mass spectrometry and N-terminal sequencing. Numerous toxins are present in treesnake venoms, including several metalloproteases, acetycholinesterases and helveprins. Additionally, MALDI-TOF and electrophoresis revealed the presence of several small toxins (8-10 kD) in the venom. A prominent metalloprotease in the venom (49.9 kD) is a member of the P-III SVMP and shows significant N-terminal sequence homology with several other SVMPs, specifically with several cloned from *Atractaspis* venom glands. High acetylcholinesterase activity is present in the crude venom, and several isoforms of the enzyme exist. A helveprin (25.2 kD) is a minor component of the venom, and it shows considerable N-terminal sequence homology with other recently described helveprins. Based on N-terminal sequence, one of the smaller toxins (9.98 kD) is a homolog of several of the weak neurotoxins present in elapid venoms. Comparative two-dimensional electrophoresis of neonate and adult snake venoms indicates a high degree of similarity of venoms from different age classes; however, metalloproteases are more abundant in the adult snake venoms. Using a combination of classical and proteomic methods, we have identified most of the major proteins in brown treesnake venoms, and we are currently conducting biological assays of select components to determine activities and biological roles of these molecules. Brown treesnake venom contains many components found in other snake venoms, and as sequence data is accumulated, inferences about the evolution of these toxins will become possible. However, it is abundantly clear that colubrid venoms are homologous with the venoms of front-fanged snakes, though compounds unique to colubrid snakes are also present. A proteomic approach to the study of colubrid venoms, coupled with more traditional toxicity and purification studies, will allow for rapid identification of the primary components of this diverse group of snakes.

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 18401 4PF.3

INTRASPECIFIC VARIATION OF A PEPTIDE IN THE BRAZILIAN MOLLUSK CONUS REGIUS VENOM.

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The carnivorous mollusk *Conus* paralyzes its prey by injecting a rich mixture of biologically active peptides. *Conus regius* is a vermivorous member of this genus that inhabits Brazilian tropical waters. Inter-, intra-species and individual variations of cone snail venom have been reported. In order to investigate intraspecific differences in *Conus* regius venom, animals were collected in Fernando de Noronha Archipelago, PE, Brazil, and pooled according to: gender, size and season of collection. Presence and quantification of one of the major components of the venom, a peptide with four disulfide cross-links (rg11a), were compared among the groups. From *C. regius* venom ducts, a cDNA library was constructed and used as template for PCR screening. Specific primers corresponding to the nucleotide sequences of signal peptide and of internal region from the mature toxin were also used. Amplified cDNA was cloned and sequenced. By PCR coupled to reverse transcription (RT-PCR), it was verified that mRNA for rg11a peptide is expressed in all groups. Quantitative analyses were performed by reverse phase HPLC and peaks were screened by MALDI-TOF mass spectrometry. Chromatography profiles revealed differences in toxin concentration among groups. Unexpectedly, variation related to gender was not observed. Season of the year seemed to be the determinant factor for the differential concentration of the peptide among the groups. In fact, during summer, this peptide is more expressed along the venom duct. This seasonal variation is observed in male; the female venom composition is more constant. This variability might be a result of environmental pressure, related to prey's offer.

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Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 18701 4PF.3

BIOACTIVE PEPTIDES FROM THE SKIN OF PRHYNOHYAS VENULOSA (HYLIDAE)

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The innate immunity is the key component of the host first defense mechanism against pathogens in organisms as diverse as plants, insects, lower vertebrates, and mammalian. More than six hundred structures with antimicrobial activity against encapsulated virus, bacteria and fungi have been compiled.

In general, a family of peptides belonging to one determined class is detected in different biological source. This results from the hypermutation of mature peptide and an extremely conserved signal peptide an indication that a high selective pressure oprerates to preserve an efficient cassette of secretion.

To identify precursors of bioactive peptides in the frog skin of *Phrynohyas venulosa* a Hylidae that inhabits the Brazilian Cerrado, a cDNA library was constructed. Based on conserved signal peptide sequence, oligonucleotides were synthetized and used to screen the recombinant bacteriophage clones. By this approach, we identified, so far, three new antimicrobial/opioid pre-propeptide precursors that share some sequence similarity with dermaseptin/demorphin members, isolated by several research groups, from the skin of Phyllomedusa specimens.

Skin secretion was fractionated by reverse phase HPLC to identify the antimicrobial fractions. Microbial inhibition growth of some fractions were observed by minimum inhibitory concentration assay.

The biochemical and molecular evolutionary aspects of these peptides are presented and discussed.

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 18901 4PF.3

TETRODOTOXIN-PRODUCING ABILITY OF BACTERIA ISOLATED FROM THE RIBBON WORM CEPHALOTHRIX SP. (NEMERTEA) IN HIROSHIMA BAY, HIROSHIMA PREFECTURE, JAPAN

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One gram of live specimen of ribbon worm *Cephalothrix* (Nemertea), containing extremely high content of tetrodotoxin (TTX), was collected in Hiroshima Bay, Hiroshima Prefecture, Japan, in May, 2003. Intestinal contents were examined for bacterial flora by the routine procedure using PYBG agar medium. A total of 40 strains were isolated by this procedure. Among these strains, twenty-eight strains growing well both in PYBG medium and in 1%NaCl and phytone peptone liquid medium were isolated. One strain (N4-2-46) out of them was cultivated in 2,000ml of the 1%NaCl and phytone peptone liquid medium at 27°C for 24days. Cells of the strain (N4-2-46) were harvested by centrifugation and ultrasonicated with 0.1% AcOH, and the extract assayed for lethal potency. Results showed that only a little potency to mice was detected in 24 day-culture. TTX fraction was partially purified from the extract by ultrafiltration (<3,000 dalton), activated charcoal and Bio-Gel P-2 column chromatography, and analyzed for TTXs using GC-MS, HPLC and LC-TOFMS. In GC-MS analysis of the alkali-hydrolyzate of this extract, mass fragment ion peaks at m/z 376, 392 and 407, which are characteristic of the quinazoline skeleton (C9-base), appeared at almost the same retention time. On the other hand, major component having the retention time close to that of TTX in HPLC analysis showed (M+H)+ and (M+H-H2O)+ at m/z 320 and 302, respectively, by LC-TOFMS. This strain produced TTX and/or related substances, as demonstrated by instrumental analyses. It was suggested from these results that the bacteria are closely associated with toxification of this ribbon worm. Identification of this strain (N4-2-46) is now under progress.

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 20101 4PF.3

ANALYSIS OF TETRODOTOXIN IN SERUM USING HIGH PRESSURE LIQUID CHROMATOGRAPHY

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Although a rare clinical problem, puffer fish poisoning can be severe and continues to cause fatalities in southeast Asia and Australia. Currently there are no simple and accessible methods for detecting or quantifying tetrodotoxin (TTX) in patients with poisoning. Availability of serum quantification of TTX would allow better case definition and investigation of the pharmacokinetics of TTX in poisoned patients. The objective of this study was to develop an easy but sensitive method for detecting TTX in human serum.

Previously published methods have used immunoaffinity chromatography , or the conversion of TTX to its C9-base derivative for measurement by mass spectrometry [1]. This method uses high pressure liquid chromatography (HPLC) with post-column derivatisation and fluorescence detection. Sample preparation is rapid and involves application to a C18 Sep-Pak cartridge and cation exchange cartridge.

Chromatograms of commercially obtained standards of TTX show two peaks, thought to be different analogues of TTX. The ability to measure both peaks in plasma samples may be significant. Little, if any, information is available on the relative potencies of the different analogues and it is unclear what forms occur in human poisoning. This method allows detection of total TTX compounds at a level of 0.1ug/ml as well as separation of analogues using a simpler and less expensive method.

1. Hayashida et al (Jap.J.Forensic Toxicol., 2002, 20, 188

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 21903 4PF.3

PURIFICATION AND CHARACTERIZATION OF TWO PLATELET AGGREGATION-INHIBITORY PHOSPHOLIPASES A_2 FROM BOTHROPS ERYTHROMELAS (JARARACA MALHA DE CASCAVEL) SNAKE VENOM.

Modesto, J.C.A[1].; Schattner, M.[2]; Spencer, P.J.[3]; Oliva, M.L.V.[4]; Chudzinski-Tavassi, A.M.[1]; Sampaio, C.A.M.[4] and Guarnieri, M.C[5].

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Two phospholipase A_2, namely Bery-I-PLA_2 and Bery-II- PLA_2, were purified from *Bothrops erythromelas* venom by successive chromatography on Superdex 75, Mono Q and Vydac-C4 columns. They have molecular weight of 13649.57 ± 0.18 and 13650.1 ± 0.23 as determined by mass spectrometry N-terminal sequences were SLVQFETLIMKIAGRSGVWY and SLVQFETLIMKIAGA, respectively, showing high degree of homology with PLA_2 from *B. pictus* venom. Both purified PLA_2 had high catalyitc activity. Althougth both toxins inhibited platelet aggregation, due to the low yield of Bery II PLA_2, it could not be used for further characterization. Bery-I-PLA_2 (1,25μg-5μg) showed an inhibitory effect on platelet aggregation induced by collagen and arachidonic acid in human platelet-rich plasma (PRP) in a dose-dependent manner, whereas it did not affect platelet aggregation with ADP as the agonist. When Bery-I-PLA_2 was chemically modified with p-Bromophenacyl bromide, its enzymatic activity and inhibitory effect on collagen-induced aggregation were abolished, while the arachidonic acid-induced aggregation was little affected. Furthermore, studies with flow cytometry showed that Bery-I-PLA_2 did not affect the surface GPIb, GPIIb and P-selectin. In studies performed using washed platelet, purified enzyme did not inhibit aggregation in response to collagen. Addition of normal plasma (50μl) to Bery-I-PLA_2 partially restored the inhibitory effect of the enzyme. These preliminaries studies suggest that the inhibitory action of Bery-I-PLA_2 on collagen-induced aggregation is dependent of their enzymatic generation of lysophosphatidylcholine from plasma lipoproteins, and that this product did not interfere with the membrane receptors analyzed. On the other hand, experiments with arachidonic acid as agonist showed dissociation between enzymatic and pharmacological activities. The molecular basis for this effect has not been established.

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 23701 4PF.3

PURIFICATION AND CHARACTERISATION OF THE FACTOR XA-LIKE PROTEASE FROM THE AUSTRALIAN COMMON BROWN SNAKE (PSEUDONAJA TEXTILIS)

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The venom from *Pseudonaja textilis* (the Australian common brown snake) contains a powerful prothrombin activator complex which is similar in structure and function to the prothrombinase complex found in mammalian blood. The enzyme within the prothrombin activator complex from *P. textilis* has been identified as a Factor Xa-like protease. Characterisation of the snake's prothrombin activator complex, the active protease within the complex and the manner in which the components interact will provide insight into why the venom is such an efficient procoagulant. The data presented here describes the chromatographic purification and the structural and catalytic characterisation of the Factor Xa-like protease from the venom of *P. textilis*.

Poster Session 4PF.3 - Glass Foya - 1100-1140

ABSTRACT NUMBER 23801 4PF.3

EXPRESSION, REFOLDING AND IN VITRO ACTIVATION OF A RECOMBINANT FACTOR XA-LIKE PROTEASE DERIVED FROM THE VENOM OF PSEUDONAJA TEXTILIS (AUSTRALIAN COMMON BROWN SNAKE)

Naomi L. Perry [1], Igor Filoppovich [2], Natasha Sorokina [2], Paul P Masci [1] and Martin F. Lavin [2]

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A number of animal species have evolved potent venoms as a defence mechanism or to immobilise prey. One of the most significant effects of some venoms is the disruption of mammalian haemostasis. Several proteins have been identified from various snake venoms that activate prothrombin to thrombin (prothrombin activators), resulting in blood coagulation. One such prothrombin activator was identified in the Australian common brown snake (*Pseudonaja textilis*); in particular, the factor Xa-like protease component of the complex has been shown to cleave a chromogenic substrate specific for human factor Xa and to clot normal human citrated plasma in vitro. Like human factor Xa, this protease is comprised of a heavy and a light chain joined by a single disulphide bond with a total of eleven disulphide bonds in the molecule. We describe the cloning of this Factor Xa-like protease from *P. textilis* and its expression in *E. coli* as inclusion bodies, from which the recombinant protease was refolded and activated in vitro by affinity processing. We also describe the expression of the recombinant protease in a mammalian cell culture system. Recombinant factor Xa-like protease may be used to stem blood loss from a wound or during surgery.

FRIDAY September 19th

SCIENTIFIC PROGRAMME

Plenary Lecture Session 5AH - Hall C - 0845-1030

Session Chairperson: Herve Rochat

Redi Award Ceremony
Redi Award Lecture

5AH.1 Shin-Ho Chung
MODELING OF ION CHANNEL GATING AND PERMEATION

Poster Session 5PF.4 - Glass Foya - 1100-1140

Poster Abstracts can be found at the end of abstracts for Friday

Invited Lecture Session 5BH - Hall C - 1140-1300 - Clinical Toxinology

Session Chairperson: David Warrell

5BH1 RDG Theakston
BIODETECTION USING ENZYME IMMUNOASSAY

5BH2 Richard C. Dart
INITIAL CLINICAL EXPERERIENCE WITH POLYVALENT CROTALID ANTIVENIN (OVINE) FAB: DO CLINICAL TRIALS REFLECT WIDESPREAD CLINICAL USE?

5BH3 G.K. Isbister
SPIDER MYTHOLOGY AND DATA COLLECTION IN CLINICAL TOXINOLOGY.

Invited Lecture Session 5BM - Meeting Room 1&2 - 1140-1300 - ASB Session - Protein Structure & Interaction

Session Chairperson: David Saint

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POST TRANSLATIONAL MODIFICATIONS

5BM2 Michael Parker
INSIGHTS INTO RECEPTOR SIGNALLING REVEALED BY CRYSTALLOGRAPHIC STUDIES

5BM3 Chris Bagely
PROTEIN STRUCTURE

Oral Papers Session 5CH - Hall C - 1400-1530 - Structure/Function of Toxins

Session Chairperson: Andrez Menez

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PROTEOMIC VISUALIZATION OF VIPERID VENOMS: COMPLEXITY, DIVERSITY AND SIMILARITY.

5CH2 T Ogawa
SERUM INIHIBITORS AGAINST PHOSPHOLIPASES A2 IN TRIMERESURUS FLAVOVIRIDIS SNAKE.

5CH3 Patrick Spencer
CLONING SEQUENCING AND EXPRESSION OF BOTHROPSTOXIN-1, A K49 MYOTOXIN

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INFLUENCE OF NON-CATALYTIC SITE RESIDUES ON CATALYTIC FUNCTION OF TYPE II PLA2

5CH5 GM Nicholson
ISOLATION AND CHARACTERISATION OF A NOVEL INSECT-SELECTIVE NEUROTOXIN FROM THE VENOM OF THE FEMALE AUSTRALIAN EASTERN MOUSE SPIDER (MISSULENA BRADLEYI)

5CH6 MM Thwin
PLA2 INHIBITORY PEPTIDE DERIVED FROM PYTHON RETICULATUS SERUM MODULATES INFLAMMATORY ARTHRITIS IN TRANSGENIC MICE

Oral Papers Session 5CM - Meeting Room 1&2 - 1400-1530 - ASB Session - Ion Channels

Session Chairperson: to be announced

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STRUCTURAL AND FUNCTIONAL DISSECTION OF THE MECHANOSENSITIVE CHANNEL MSCL

5CM2 Brown
INSERTION OF THE CHLORIDE ION CHANNEL 'CLIC1' INTO PHOSPHOLIPID MEMBRANES

5CM3 Barry
MUTANT GLYCINE RECEPTOR CHANNELS, WITH REVERSED ION CHARGE SELECTIVITIES, AND ANALYSIS OF THEIR ANION-CATION PERMEABILITES

5CM4 Rychkoy
MECHANISM OF THE FAST INACTIVATION OF THE CA^{2+} RELEASE ACTIVATED CA^{2+} CHANNEL

Oral Papers Session 5CR - Meeting Room 3 - 1400-1530 - Antivenoms

Session Chairperson: Julian White

5CR1 RDG Theakston
CRISIS IN SNAKE ANTIVENOM SUPPLY FOR AFRICA: IS THERE A SOLUTION?

5CR2 D.A. Warrell
RANDOMISED, BLINDED COMPARISON OF THREE POLYSPECIFIC ANTIVENOMS IN THE TREATMENT OF SYSTEMIC ENVENOMING BY BOTHROPS ATROX AND B. BILINEATA IN THE ECUADOREAN AMAZON REGION.

5CR3 Kenneth D Winkel
ENZYMATIC CHARACTERIZATION, ANTIGENIC CROSS-REACTIVITY AND NEUTRALIZATION OF DERMONECROTIC ACTIVITY OF MEDICALLY IMPORTANT LOXOSCELES SPIDER VENOMS

5CR4 Kavi Ratanabanangkoon
HYALURONIDASE INHIBITORS (SODIUM CROMOGLYCATE AND SODIUM AURO-THIOMALATE) REDUCE THE LOCAL TISSUE DAMAGE AND PROLONG THE SURVIVAL TIME OF MICE INJECTED WITH NAJA KAOUTHIA AND CALLOSELASMA RHODOSTOMA VENOMS

5CR5 Kiem Trinh Xuan
THE PRODUCTION OF CALLOSELASMA RHODOSTOMA ANTIVENOM (CR-AV) FROM EGG YOLKS OF HENS IMMUNIZED WITH VENOM AND ITS APPLICATION FOR THE TREATMENT OF SNAKEBITE PATIENTS IN VIETNAM

5CR6 J. White
ENVENOMING BY THE HOPLOCEPHALUS GENUS (ELAPIDAE: AUSTRALIA)

Oral Papers Session 5DH - Hall C - 1600-1700 - Clinical Round Table

Session Chairperson: Julian White

The precise format for this session was not available at time of printing the Abstract Book. Details will be made available during the Congress.

Oral Papers Session 5DM - Meeting Room 1&2 - 1600-1730 - ASB Session - Protein Structure/ Function

Session Chairperson: to be announced

5DM1 Moens
DIMERIZATION MODE OF *T. MARITIMA* RIBOSOMAL STALK PROTEINS L12 IN SOLUTION

5DM2 Coster
INSULIN DEPENDENCE OF GLUCOSE TRANSPORTER GLUT4 IN ADIPOCYTES

5DM3 Gready
CONFORMATION OF PRION PROTEIN REPEAT PEPTIDES PROBED BY FRET MEASUREMENTS AND MD SIMULATIONS

5DM4 Klonis
FLUORESCENCE PHOTOBLEACHING TECHNIQUES ON THE CONFOCAL MICROSCOPE FOR THE EXAMINATION OF PROTEIN AND CELLULAR DYNAMICS

FRIDAY September 19th

IST Redi Award Lecture - Hall C - 0900-0950

The Redi Award Lecture is presented each IST World Congress by the Award Recipient for the Congress. The Award is announced at the Congress, therefore the title and content of this lecture are not available prior to the Award Ceremony and are not present in the Abstract Book.

Australian Society of Biophysics Plenary Lecture - Hall C - 0950-1030

NO ABSTRACT AVAILABLE

MODELING ION CHANNEL GATING AND PERMEATION

Shin-Ho Chung

Invited Lecture 5BH.1 - Hall C - 1140-1210

ABSTRACT NUMBER 07001 5BH1

BIODETECTION USING ENZYME IMMUNOASSAY

RDG Theakston

Alistair Reid Venom Research Unit, Liverpool School of Tropical Medicine, Liverpool, UK.

The major roles of enzyme immunoassay in venom research are in (1) accurate retrospective diagnosis, (2) assessing new and existing antivenoms, (3) examining the effectiveness of first-aid measures, (4) detecting specific antibody in previously envenomed victims and (5) detecting levels of individual venom components. In most cases of envenoming, the snake responsible for the accident is not identified and EIA has proved useful for identifying the snake causing the accident. However, the main problem, which has not been overcome, is the time taken to provide a diagnosis which is too long to assist the clinician in treating the patient with the correct monospecific antivenom. Historically antivenoms are first assessed using in vivo rodent and in vitro tests which determine whether the antivenom is effective preclinically. Antivenoms which pass this test are then tested clinically to see whether they are capable of reversing the signs of systemic envenoming (eg, haemorrhage, coagulopathy, neurotoxicity). Whereas in the past, treatment of patients has depended almost entirely on the individual clinician's experience in assessing the identity of the snake and the severity of envenoming, the development of EIA has enabled a more scientific appraisal of the situation by permitting the accurate estimation of levels of circulating specific venom and antivenom at any time in the patient's blood or other body fluids. It is therefore possible to measure the efficacy of antivenom in clearing venom antigen. In Brazil, it has been shown that clinicians are probably treating patients with excessive amounts of highly efficient *Bothrops* polyspecific antivenom, with a resulting unacceptably high incidence of early and delayed reactions and in Sri Lanka the use of imported Indian antivenom is relatively ineffective in neutralizing the venom of Sri Lankan *Daboia russelii*, demonstrating the real problem of venom variability within individual species. Such clinically-based immunological studies provide an objective assessment of antivenom therapy which has not in the past been feasible. This should result in a more efficient and controlled use of expensive antivenoms for treatment of systemic envenoming. Such studies also emphasize the importance of individual countries producing their own antivenoms for treatment of snakebite. The use of the method as an epidemiological tool by which specific venom antibodies may be detected requires further investigation. EIA can also be used for assessing the value of traditional and new first aid measures such as tourniquets and the possible use of small fragment antivenoms, admistered by the intramuscular route, as a possible prehospital treatment for envenoming. In addition, EIA has application in the pharmacokinetic study of important venom components in both humans and experimental animals. The search for a more rapid immunodiagnostic system continues.

Invited Lecture 5BH.2 - Hall C - 1210-1235

ABSTRACT NUMBER 13101 5BH2

INITIAL CLINICAL EXPERERIENCE WITH POLYVALENT CROTALID ANTIVENIN (OVINE) FAB: DO CLINICAL TRIALS REFLECT WIDESPREAD CLINICAL USE?

Richard C. Dart, MD, PhD.

Rocky Mountain Poison and Drug Center, Denver Health, and University of Colorado Health Sciences Center, Denver, Colorado

Polyvalent Crotalid Antivenin (Ovine) Fab (CroFab; FabAV) was introduced in the United States in 2001. The basis for FDA approval was two small studies enrolling a total of 42 patients. Thus, there is much to be learned about the use of this drug in clinic practice. FabAV is produced against the venoms of *Crotalus adamanteus, C. atrox, C. scutulatus scutulatus* and *Agkistrodon piscivorus*. The immune serum produced in sheep is processed to contain highly purified Fab only.

Postmarketing surveillance has revealed unexpected usage patterns as well as solidified understanding of the adverse events associated with this product. The efficacy of FabAV when administered within the first few hours of snakebite has been excellent. The unusual dosing schedule of FabAV has produced some confusion when a practitioner uses it for the first time. Data regarding the use of Fab AV in children and for new snake species will be provided.

Another important issue that has developed involves the concept of recurrence. Defined as the return of venom effects after they had been under documented clinical control, recurrence can occur in local wound effects, coagulation abnormalities and in systemic signs. To date, there have been no instances of systemic recurrence reported. However, both local recurrence and coagulation recurrence have been described. Data regarding the incidence and geographic distribution of this phenomenon will be presented.

The finding of coagulation recurrence challenges the clinical dogma in the United States regarding the use of coagulopathy as an indication for antivenom treatment. To date, no episodes of spontaneous bleeding in patients with severe coagulation recurrence have been reported, despite nearly unmeasurable fibrinogen and severe thrombocytopenia in some cases. This data will be summarized and the analysis provided on the appropriate management of patients with recurrence following the use of FabAV.

The implications for the clinical management of snakebite in the United States are large. To alter the prescribing behavior of a wide variety of physicians throughout large portions of the country is a difficult task. However, it is likely that any antivenom will demonstrate the phenomenon of recurrence as evidenced by reports from a wide variety of snake species. Therefore, a management strategy that includes monitoring, re-evaluation and possibly retreatment is needed.

Invited Lecture 5BH.3 - Hall C - 1235-1300

ABSTRACT NUMBER 14203 3BM2

SPIDER MYTHOLOGY AND DATA COLLECTION IN CLINICAL TOXINOLOGY.

G.K. Isbister

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Clinical toxinology suffers from a long history of poor data collection. A brief review of MEDLINE illustrates the lack of randomised controlled trials and prospective studies in clinical toxinology. Mythology surrounds bites and stings by particular creatures, resulting from the general fear of many creatures, such as spiders, which has not been disproved by appropriate well designed studies. The current focus on necrotic arachnidism in many parts of the world is a good example. Previously, most studies have been retrospective, bites and stings not confirmed and creatures not kept or incorrectly identified. Prospective observational studies of confirmed bites with correct identification of the creature are required in clinical toxinology. This requires collaboration between those who can correctly identify the animals (biologists/taxonomists) and those involved in the clinical management (poison information services, emergency departments and toxicology services). Prospective collection of data pertaining to the circumstances and effects of the bites is essential. Routine follow up is required to identify delayed effects and duration of immediate effects. Analysis of databases created from prospective studies will not only answer questions about the effects of different species, but will ultimately allow development of evidence based methods to identify animals based on the circumstances and effects of bites, rather than requiring formal identification of the culprit.

Australian Society of Biophysics Invited Lecture 5BM.1 - Meeting Room 1&2 - 1140-1210

NO ABSTRACT AVAILABLE

POST TRANSLATIONAL MODIFICATIONS

Dick Wettenhall

Dept of Biochemistry and Molecular Biology, University of Melbourne

Australian Society of Biophysics Invited Lecture 5BM.2 - Meeting Room 1&2 - 1210-1235

NO ABSTRACT AVAILABLE

INSIGHTS INTO RECEPTOR SIGNALLING REVEALED BY CRYSTALLOGRAPHIC STUDIES

Michael Parker

Biota Structural Biology Laboratory, St. Vincent's Institute of Medical Research, Melbourne, Victoria

Australian Society of Biophysics Invited Lecture 5BM.3 - Meeting Room 1&2 - 1235-1300

NO ABSTRACT AVAILABLE

PROTEIN STRUCTURE

Chris Bagely

Head, Protein Laboratory, Hanson Institute, IMVS, Adelaide SA

Structure/Function of Toxins - Hall C - 1400-1415

ABSTRACT NUMBER 07403 5CH1

PROTEOMIC VISUALIZATION OF VIPERID VENOMS: COMPLEXITY, DIVERSITY AND SIMILARITY.

Solange M.T. Serrano[1], John D. Shannon[2], Deyu Wang[2], Antonio C.M. Camargo[1], Jay W. Fox[2].

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2 Biomolecular Research Facility, Department of Microbiology, University of Virginia Health System, Charlottesville, VA 22908-0734. USA.

The complexity of viperid venoms has long been appreciated in the field of toxinology and medicine. However, it is only recently that the depth of that complexity has become quantitatively accessible. With the resurgence of 2D PAGE and the advances in mass spectrometry essentially all venom components can be visualized and identified given the effort and resources. Here we present the use of various technologies for examining venom complexity and demonstrate their associated advantages and disadvantages. 2D PAGE comparisons between different genera of viperid venoms; different species of the same genus and specimens of the same genus and species demonstrate the similarity as well as the apparent diversity among these venoms. Finally, we have applied techniques to examine subpopulations of venom proteins (metalloproteinase proteome; serine proteinase proteome; phospholipase A2 proteome and the glycoproteome). Subsequently, we identified antigenic venom components using polyvalent anti-Crotalus antiserum. These tools will allow for a better understanding of venom complexity and toxic properties as well as enabling investigators with particular interests to focus on these subpopulations of proteins for further study.

Structure/Function of Toxins - Hall C - 1415-1430

ABSTRACT NUMBER 12701 5CH2

SERUM INIHIBITORS AGAINST PHOSPHOLIPASES A_2 IN TRIMERESURUS FLAVOVIRIDIS SNAKE.

T Ogawa [1], K Yoneyama [1], Y Akahori [1], K Muramoto [1], M Kitano [2], S Hattori [3], M Ohno [4]

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Many venomous snakes are resistant to their own venoms due to the presence of neutralizing factors in their sera. To date, snake venom PLA_2 inhibitors (PLIs) have been isolated from the various snake sera and their primary structures have been determined. Two inhibitors (PLI-A and PLI-B) against [Asp49]PLA_2, which contain a C-type carbohydrate recognition domain (CRD)-like motif and are classified into PLIa, and myotoxic [Lys49]PLA_2 (BPII)-binding protein (PLI-I) that has two three-fingered motifs and belongs to PLIg (Ly-6 superfamily), were isolated from Trimeresurus flavoviridis serum. However, the effects of these inhibitors against myotoxic PLA2s and their inhibitory mechanism have not been elucidated. In the present study, anti-myonecrotic activities of these inhibitors against [Lys49]PLA2 and [Asp49]PLA_2 were assessed by using cultured cells (BHK21 and SkMC). PLI-I showed inhibitory activity against BPII ([Lys49]PLA_2)-induced myonecrosis but not against [Asp49]PLA_2-induced necrosis. Although PLI-A and PLI-B were isolated as [Asp49]PLA_2 inhibitors, they also showed more potent inhibitory activity against BPII-induced myonecrosis than PLI-I. PLI-A lacking N-terminal 37 amino acids lost its inhibitory activity, suggesting that N-terminal region is crucial for inhibitory activity. In addition, we constructed recombinant expression system of PLI-A and PLI-B to elucidate the structure-activity relationships. Based on these results, the inhibitory mechanism of PLIs for myonecrosis will be discussed.

Structure/Function of Toxins - Hall C - 1430-1445

ABSTRACT NUMBER 21902 5CH3

CLONING SEQUENCING AND EXPRESSION OF BOTHROPSTOXIN-1, A K49 MYOTOXIN

Spencer, P.J. [1]; Webb, R.P. 2; Lomonte, B. 3; Angulo, Y. [3]; Campos, L.A. [1]; Moura da Silva, A. M. [4] & Smith, L.A. [2] .

1-Laboratório de Química de Proteínas, Centro de Biologia Molecular, IPEN/CNEN, São Paulo, Brazil. 2- Toxinology and Aerobiology Division US Army Medical Research Institute for Infectious Diseases, Frederick, MD, USA. 3- Instituto Clodomiro Picado, Facultad de Microbiologia, Universidad de Costa Rica, San José, Costa Rica. 4- Laboratório de Imunopatologia, Instituto Butantan, São Paulo, Brazil.

With the aim of obtaining recombinant Bothropstoxin-1 for functional studies, we constructed a cDNA library wich was then screened with a specific probe. A positive clone was then circularized and sequenced, yielding previously unreported 5'and 3' untranslated regions, a 48 bp domain encoding for signal peptide and the mature protein encoding region. The deduced aminoacid sequence was in complete agreement with the previously obtained by Edman degradation. The open reading frame for the toxin was subcloned in the pET 24 expression vector and transformed in BL21 E. coli. After induction, the recombinant product was obtained as inclusion bodies, refolded and purified by ion exchange chromatography. Nor electrophoresis analysis neither reverse-phase chromatography revealed any differences between the native and recombinant toxins. When assayed for activity in vivo and in vitro, no differences were observed between the native toxin and its recombinant counterpart. This might represent a first step towards obtaining properly refolded mutants for structure-function studies.

Structure/Function of Toxins - Hall C - 1445-1500

ABSTRACT NUMBER 13601 5CH4

STUDY OF THE MECHANISM OF ACTION OF SNAKE PRESYNAPTIC PLA_2 NEUROTOXINS

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Several animal venoms contain toxins with phospholipase A_2 (PLA_2) activity. These enzymes hydrolyse the sn-2 ester bond of 1,2-diacyl-3-sn-phosphoglycerides producing fatty acids and lysophospholipids. Some snake venoms contains presynaptic PLA_2 neurotoxins that cause a persistent blockade of neurotransmitter release from nerve terminals. Three subsequent phases can be distinguished at the neuromuscular junction (NMJ) poisoned by PLA_2 neurotoxins: a short initial phase with either decreased or unchanged ACh release, is followed by a longer phase of facilitation of ACh release, which then fades into the third phase of complete and irreversible inhibition of neurotransmission. Electron microscopy studies of poisoned NMJ revealed appearance of many clathrin-coated omega-shaped plasma membrane invaginations, indicating a blockage of endocytosis. Therefore PLA_2 neurotoxins both promote fusion of small synaptic vesicles (SSVs) with the presynaptic membrane and inhibit their retrieval, thus causing release of ACh, depletion of vesicles and enlargement of nerve terminals. We are studying the effect of four snake presynaptic neurotoxins of different structural complexity on various primary neuronal cultures. The toxins used were the single chain notexin, the double chain beta-bungarotoxin, the trimeric taipoxin and the pentameric textilotoxin. Cerebellar granule, cortical and hippocampal neurons in culture were examined by light and electron scanning microscopy after exposure for 1 hr with nanomolar toxin concentrations and the distribution of cytoskeletal, vescicular and membrane markers were analysed by immunofluorescence. The data obtained will be discussed in terms of the characterisation of primary neuronal cultures as pertinent models for studying the mechanism of action of snake presynaptic neurotoxins and indicate that these toxins are interesting tools to study synaptic vesicle fusion and recycling.

Structure/Function of Toxins - Hall C - 1500-1515

ABSTRACT NUMBER 07301 5CH5

ISOLATION AND CHARACTERISATION OF A NOVEL INSECT-SELECTIVE NEUROTOXIN FROM THE VENOM OF THE FEMALE AUSTRALIAN EASTERN MOUSE SPIDER (MISSULENA BRADLEYI)

Y Chong[1], AA Khalife[1], PG Hains[2], KW Broady[3], GM Nicholson[1]

Departments of 1Health Sciences and 3Cell & Molecular Biology, University of Technology Sydney, NSW 2007, Australia; 2Department of Chemistry, University of Wollongong, NSW 2522, Australia

Biological pesticides, such as recombinant baculoviruses, that specifically target pest insects are a promising alternative to agrochemicals, but require the insertion of a known insecticidal toxin gene from natural sources to maximise their efficacy. This project aimed to identify an insect-selective toxin from the venom of the Eastern mouse spider (Araneae: Mygalomorphae: Actinopodidae) for possible future recombinant baculoviral applications. Mouse spiders are a novel source of toxins since they are taxonomically distinct from Australian funnel-web spiders, the most extensively studied source of insecticidal neurotoxins. The venom from the female Eastern mouse spider was collected and fractionated using C18 reverse-phase HPLC. Several crude peptide fractions were screened by lateroventral injection for insecticidal activity in House crickets (*Acheta domesticus*) at a dose of 10 μg/g. The most toxic of these fractions showed signs of irreversible flaccid paralysis, with death occurring within 90 minutes post-injection. This fraction was further purified using anion-exchange chromatography, and the isolated peptide was found to have a molecular weight of 4167 ± 1 Da as determined by MALDI-TOF mass spectrometry. The median lethal dose (LD_{50}) and median knockdown dose (KD_{50}) at 48 hours post-injection in crickets was determined to be 50 ± 4 pmol/g and 35 ± 2 pmol/g respectively. It was then confirmed that the purified toxin lacked vertebrate toxicity in bioassays using an isolated chick biventer cervicis nerve-muscle preparation at concentrations up to 1 mM. N-terminal sequencing revealed a 39-residue peptide (Swiss-Prot accession number - P83588) with sequence homology to a family of 37-residue insecticidal Ca^{2+} channel blocking toxins (ω-ACTX-1) from the venom of funnel-web spiders. Apart from variations in the peptide backbone between cysteines IV and V (Cys19 and Cys30), the remaining four cysteines are virtually identical in spacing with the ω-ACTX-1 family. Consequently, high degrees of homology were noted only within the N- and C-terminal regions of this family (overall 51% identity and 56% homology). Nevertheless, the known pharmacophore of ω-ACTX-Hv1a (Gln9, Pro10, Asn27, Arg35) is conserved. Given the homology to the ω-ACTX-1 family, this toxin was named ω-missulenatoxin-Mb1a (ω-MSTX-Mb1a) based on the nomenclature for funnel-web spider toxins. Current experiments are aimed at confirming that ω-MSTX-Mb1a blocks insect voltage-gated Ca^{2+} channels.

Structure/Function of Toxins - Hall C - 1515-1530

ABSTRACT NUMBER 17201 5CH6

PLA_2 INHIBITORY PEPTIDE DERIVED FROM PYTHON RETICULATUS SERUM MODULATES INFLAMMATORY ARTHRITIS IN TRANSGENIC MICE

MM Thwin [1], E Douni [2], V Aidinis [2], G Kollias [2], K Sato [3], P Gopalakrishnakone [1]

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PIP, the phospholipase A_2 (PLA_2) inhibitor from *Python reticulatus* serum, neutralizes the lethal potency of snake venom PLA_2s, and modulates pro-inflammatory potency of mammalian secretory(s) PLA_2. We have designed a peptide antagonist of $sPLA_2$ based on database alignment of primary sequences of PIP and related snake blood-based PLA_2 inhibitors (PLIs). This study aims to evaluate the efficacy of prophylatic administration of PLA_2 inhibitory peptide P-NT.II in suppressing progressive development of joint inflammation in transgenic (Tg197) mice carrying human TNFa transgenes. Intraperitoneal administration of P-NT.II for 5-weeks in groups of Tg197 mice, significantly attenuated the onset and development of chronic joint inflammation as evidenced by reduced arthritic indices and histopathological scores. By light and transmission electron microscopic examinations, we observed reduction in joint inflammation and swelling, an apparent suppression of pannus development, and minimal erosive damage to the articular cartilage and subchondral bone in the knee joints of peptide-treated Tg197 mice. The elevated levels of circulating $sPLA_2$ usually found at 5th week in untreated Tg197 mice, were significantly ($P < 0.01$) reduced in P-NT.II-treated mice, whereas serum PLA_2 levels in those treated with either the vehicle dimethylsulfoxide (DMSO) or the (negative control) peptide with scrambled sequence, remained elevated. In vitro experiments using lipopolysaccharide (LPS)-induced cultured J774 mouse macrophage cells suggest that this attenuation in joint inflammation may be associated with inhibition of arachidonic acid bioavailability through a dual inhibitory effect of P-NT.II on $sPLA_2$ activity and generation of TNFa from LPS-stimulated macrophages. Our data suggest that this novel peptide P-NT.II may be effective in the treatment of acute joint inflammation associated with rheumatoid arthritis in humans.

Australian Society of Biophysics Session - Meeting Room 1&2 - 1400-1420

ASB ABSTRACT NUMBER ASB01 5CM1

STRUCTURAL AND FUNCTIONAL DISSECTION OF THE MECHANOSENSITIVE CHANNEL MSCL

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MscL is a bacterial mechanosensitive channel gated by membrane tension in the lipid bilayer alone (Häse *et al., J. Biol Chem.* 270:18329-18334, 1995). Its structure, known from X-ray crystallography, indicates that it is a homopentamer (Chang *et al., Science* 282:2220-2226, 1998). Each sub-unit comprises two transmembrane segments TM1 and TM2 connected by a periplasmic loop. The closed pore is lined by five TM1 helices. We expressed and purified two halves of the protein in *E. coli*. Each half contained one of the transmembrane segments. The electrophysiological activity of the two transmembrane segments was studied by the patch-clamp recording upon reconstitution in artificial liposomes. The TM2 moiety had no electrophysiological activity while the TM1 half formed channels, which were not affected by membrane tension and varied in conductance between 50 and 350 pS in 100 mM KCl. Co-reconstitution of the two halves of MscL however, yielded mechanosensitive channels having the same conductance as the native MscL (1500 pS), but exhibiting increased sensitivity to pressure. This unexpected however, highly interesting result is consistent with the results of a previous study in which we examined the contributions of the extramembranous parts of the channel in mechanosensation by direct application of proteases during patch-clamp experiments performed on MscL reconstituted in artificial liposomes (Ajouz et al., *J. Biol. Chem.* 275:1015-22, 2000). In these experiments, the channel retained its native orientation with the NH2 and COOH termini facing the recording chamber and the periplasmic loop facing the interior of the patch pipette. When trypsin or chymotrypsin were present in the pipette, we observed that the sensitivity of the MscL channels increased over time. The interpretation of this observation was that the periplasmic loop had been proteolysed and that its integrity was not essential for mechanosensation, but that its cleavage enhanced mechanosensitivity. The experiments reported here clearly demonstrate this proposal and indicate that the loop plays an important role in MscL gating possibly by acting as a spring, which sets the level of mechanosensitivity of the channel.

The results of the present study confirm the current view on the functional role of TM1 and TM2 helices in the MscL gating and emphasize the importance of helix-helix interactions for the assembly and functional properties of the channel protein. In addition, the results indicate a crucial role of the periplasmic loop for the channel mechanosensitivity.

Australian Society of Biophysics Session - Meeting Room 1&2 - 1420-1440

ASB ABSTRACT NUMBER ASB02 5CM2

INSERTION OF THE CHLORIDE ION CHÁNNEL 'CLIC1' INTO PHOSPHOLIPID MEMBRANES

Louise J. Brown*, Dene R. Littler*, Samuel N. Breit# and Paul M.G. Curmi*#

**School of Physics, University of New South Wales, NSW 2052 and #Centre for Immunology, St Vincent's Hospital, Sydney NSW 2010*

Chloride ion channels are involved in diverse physiological processes and channel malfunction can lead to severe diseases. Using a combination of structural and functional studies, we are investigating a member of the newly described Chloride Intracellular Channel Family, CLIC1. CLIC1 is unique due to its ability to transit between a soluble form and an active membrane channel form.

The recently published x-ray crystallography structure from our laboratory has determined the soluble form of CLIC1 (JBC 276:44993-5000), but the membrane channel structure is unknown and is difficult to determine using traditional atomic resolution structural techniques (e.g. crystallography or NMR). Because of its size, its high levels of expression, and its ability to reconstitute into artificial bilayers, CLIC1 is an ideal candidate for detailed structural analysis using the biophysical approach of Site Directed Spin Labeling-Electron Paramagnetic Resonance Spectroscopy (SDSL-EPR). However, very little is currently known about the conditions that control the insertion of CLIC1 into the membrane bilayer or the conditions which activate the channel. Prior to detailed structural analysis of CLIC1 in the membrane bilayer using SDSL-EPR, it is essential to first determine and characterise 'environmental' parameters that may promote channel insertion and/or activation. Results from EPR and fluorescence spectroscopy, which can be used to monitor the insertion of CLIC1 into the bilayer under various parameters (e.g. pH, presence of Ca^{2+}, concentration of CLIC1 added to vesicles, role of native cysteines, length of incubation time of CLIC1 with lipid vesicles), will be presented. Additionally, the activity of the reconstituted CLIC1 channel, under the same conditions above, was determined using a 'bulk' chloride efflux assay.

Australian Society of Biophysics Session - Meeting Room 1&2 - 1440-1500

ASB ABSTRACT NUMBER ASB03 5CM3

MUTANT GLYCINE RECEPTOR CHANNELS, WITH REVERSED ION CHARGE SELECTIVITIES, AND ANALYSIS OF THEIR ANION-CATION PERMEABILITES

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Patch clamp studies of recombinant glycine receptor (GlyR) channels expressed in HEK293 cells have recently enabled us to show that a series of (single, double and triple) point mutations in the M2 region of the glycine receptor were able to each reverse the GlyR selectivity from being anion- to cation-selective, and that anion-cation selectivity is determined both by electrostatic effects and changes in minimum pore diameter at the selectivity filter of the GlyR channel (2,3,4). In order to derive the permeability ratio (P_{Cl}/P_{Na}) for each mutant GlyR channel, changes in reversal potential, ΔV_{rev}, were determined during dilutions of the external solution from a control external NaCl solution, to about 0.5 and about 0.25 of the control NaCl solutions, and these ΔV_{rev} values plotted against NaCl activities and fitted to the Goldman-Hodgkin-Katz (GHK) equation [e.g., see (1)]. We have now shown that these derived relative permeability values are fairly model independent, since fitting the data to the Planck equation with virtually opposite assumptions to the GHK equation (1), gave virtually identical (P_{Cl}/P_{Na}) values.

In addition, a three-dimensional model of the GlyR channel using Brownian dynamics (BD) simulations for the anion- and cation-selective mutant GlyRs also predicted both the changes in ion selectivity of the mutant channels and the shifts in reversal potential, which were reasonably similar to those measured experimentally (5). However, one slightly puzzling feature of the BD simulations has been that, even though the simulated reversal potentials are not equal to either the Na^+ or Cl^- Nernst equilibrium potentials, there seem to be no measurable counter ion fluxes.

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Australian Society of Biophysics Session - Meeting Room 1&2 - 1500-1520

ASB ABSTRACT NUMBER ASB04 5CM4

MECHANISM OF THE FAST INACTIVATION OF THE CA^{2+} RELEASE ACTIVATED CA^{2+} CHANNEL

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A wide variety of hormones, neurotransmitters and growth factors use Ca^{2+} as an intracellular signal to initiate cellular responses. The specificity of these responses is defined by the magnitude, duration, and location of the increase in the cytoplasmic Ca^{2+} concentration as well as by the intracellular distribution of the Ca^{2+} binding target enzymes. In nonexcitable cells such as those of immune system, endothelium and epithelium, sustained Ca^{2+} influx is mediated by store-operated channels (SOCs) among which the Ca^{2+}release activated Ca^{2+} (CRAC) channels are the best characterised. In patch-clamp recording, during hyperpolarizing pulses to potentials more negative than –80 mV CRAC channels rapidly inactivate with a double exponential time course. This inactivation is significantly reduced when Ba^{2+} is used as a charge carrier instead of Ca^{2+}, or a faster Ca^{2+} buffer, BAPTA, is used instead of EGTA, which suggests that fast inactivation of CRAC channels is mediated by the binding of Ca^{2+} close to the internal mouth of the channel [(1)].

In the present work we investigated whether a calmodulin-dependent mechanism similar to the one that regulates voltage-dependent Ca^{2+}channels is also responsible for the fast inactivation of CRAC channels in H4IIE cells, an immortalised rat liver cell line. It was found that suppression of calmodulin by over-expression of the calmodulin binding domain of type I adenylyl cyclase or a mutant form of calmodulin lacking functional EF hands significantly alters the fast inactivation of I_{CRAC}. The relative amplitude of the fast inactivating component of I_{CRAC} was decreased while the non-inactivating component increased. Kinetics of I_{CRAC} inactivation in cells where calmodulin was suppressed were similar to that of the control cells when Sr^{2+} was used instead of Ca^{2+} in the external solution or BAPTA was used in the pipette. These observations suggest that calmodulin is involved in the Ca^{2+}dependent gating of CRAC channels.

Antivenoms - Meeting Room 3 - 1400-1415

ABSTRACT NUMBER 07002 5CR1

CRISIS IN SNAKE ANTIVENOM SUPPLY FOR AFRICA: IS THERE A SOLUTION?

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The Past: In Africa, snake bites cause many hundreds of deaths each year and thousands of cases of permanent physical disability. In the past, two large European companies produced polyspecific antivenoms supplementing the production of antivenoms from South Africa. Even so, unsufficient antivenom was distributed to treat more than a fraction of African snake bite victims.

The Present: One of the European companies (Behringwerke) has stopped producing antivenom, the other (Aventis Pasteur) produces only limited amounts, while the South African company is undergoing privatisation, a threat to antivenom production which is inherently unprofitable. As a result, the burden of human suffering from snake bite in Africa is already rising and mortality is increasing. The choice is now between imported, ineffective, non-specific antivenoms manufactured in Asia using geographically inappropriate venoms and unproven, and frequently dangerous, traditional treatments. In Nigeria, relabelled expired antivenom is all that is available on the market in some areas. In the 1990s, hopes were raised by an Anglo-American company whose stated aims was to produce antivenoms for use in both developing and developed countries. Production of antivenom for use in West Africa was supported by the Nigerian Federal Ministry of Health with advice and practical help from our institutions. Welsh sheep were immunised with venom obtained from Nigerian saw-scaled vipers (*Echis ocellatus*) maintained in Liverpool. The new antivenom was assessed preclinically in Liverpool and then compared clinically with French antivenom in envenomed patients in Kaltungo, Nigeria. In that region, during the sowing and harvesting seasons, as many as 20% of hospital beds may be occupied by snake bite victims.

The Future: In 1999 the Anglo-American company merged, abandoning for financial reasons the production of antivenom for developing countries but maintaining production of antivenoms at £2000-£6000/treatment for use in Europe and North America. In 2000, an offshoot of this company was funded by two UK Research Councils and the Nigerian Government to produce economical antivenoms for Nigeria and for other developing tropical countries. Meanwhile, antivenom producers, especially those with unused production capacity, are being persuaded to manufacture antivenoms for use in Africa. It is essential that these antivenoms should be raised using venoms from African snakes. In developed regions of the world, such as Europe, Australia and USA, antivenom treatment costing thousands of pounds may be feasible, but in developing countries where snake bite takes its greatest toll of human life and limb, even treatment costing as little as £3 per vial may be neither affordable nor available.

Antivenoms - Meeting Room 3 - 1415-1430

ABSTRACT NUMBER 05403 5CR2

RANDOMISED, BLINDED COMPARISON OF THREE POLYSPECIFIC ANTIVENOMS IN THE TREATMENT OF SYSTEMIC ENVENOMING BY BOTHROPS ATROX AND B. BILINEATA IN THE ECUADOREAN AMAZON REGION.

D.A. Warrell and R.D.G. Theakston; R. Smalligan, J. Cole, B.L. Mertz, S. Manock, J. Maudlin, B. Quist, G. Holland, S. Nelson, N. Brito, G. Rivadeneira and D. G. Lalloo

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In 1993, we established that the antivenom then supplied by the Ministry of Health to hospitals throughout Ecuador; Suero antiofidico polvalente "Myn" produced by Ronti Mexico SA and distributed by Grupo Pharma S. A. de C. V., Zapata Laboratories, Mexico City; was not effective against challenge by Ecuadorean *Bothrops asper, B. atrox* and *Lachesis muta* venoms in standard WHO mouse assays.

Clinical experience in hospitals visited in Lago Agrio, Coca, Macas, Macuma and Shell confirmed this inadequacy. However, laboratory studies identified three polyspecific antivenoms manufactured by Instituto Nacional de Salud, Bogota, Colombia; Instituto Butantan, São Paulo, Brasil; and Inst. Izquieta Perez, Guayaqil, Ecuador which showed encouraging activity against the three venoms (Theakston, Laing et al. 1995).

In a series of 55 snakebite cases treated in Hospital Vozandes Oriente, Shell, Pastaza, in which the snake responsible for the bite was brought and expertly identified, 45% were caused by *B. atrox*, 38% by *B. bilineata*, 5% by *B. taeniata*, and one each by *B. pulchra, Bothrocophias hyoprora, Bc. microphthalmus, Micrurus steindachneri orcesi* and two by colubrid species (unpublished). Subsequently, a randomised, blinded comparison of three antivenoms was carried out in Shell, involving a total of 211 patients with incoagulable blood. Most had been bitten by *B. atrox* or *B. bilineata*. 87 were allocated to Colombian, 83 to Brazilian and 41* to Ecuadorean antivenom (*explained by failure of supply). There were two fatalities. The main end-point was restoration of blood coagulabilty in which the Colombian antivenom proved the most effective at 6 and 24 hours after the first dose. The mean dose of antivenom required was 4.5 (median 4; range 2-12) ampoules. 79% of patients required 6 ampoules or less.

Theakston, R. D., G. D. Laing, et al. (1995). "Treatment of snake bites by *Bothrops* species and *Lachesis muta* in Ecuador : laboratory screening of candidate antivenoms." Trans R Soc Trop Med Hyg 89(5): 550-4

Antivenoms - Meeting Room 3 - 1430-1445

ABSTRACT NUMBER 02301 5CR3

ENZYMATIC CHARACTERIZATION, ANTIGENIC CROSS-REACTIVITY AND NEUTRALIZATION OF DERMONECROTIC ACTIVITY OF MEDICALLY IMPORTANT LOXOSCELES SPIDER VENOMS

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Loxosceles spiders have a wide distribution in temperate and tropical regions of the world. The envenomation syndrome, Loxoscelism, is characterized by necrotic skin ulceration at the bite site and, less commonly, a systemic illness that may be fatal. The venom is a complex mixture of components with toxic and/or enzymatic activities. The aim of this work was to characterize and compare some aspects of the major medically important *Loxosceles* spider venoms, particularly their neutralisation by Brazilian antivenom. By SDS-PAGE, *L. deserta, L. gaucho, L. intermedia. L. laeta* and *L. reclusa* venoms showed similar electrophoretic profiles with the major bands located around 32 kDa (L. deserta and L. laeta venoms) and 35 kDa (*L. gaucho, L. intermedia* and *L. reclusa* venoms). The venoms also had several minor components between 82-220 kDa. All venoms exhibited gelatinolytic, caseinolytic and hyaluronidase activities in vitro with a large array of caseinolytic and gelatinolytic proteases mainly between 20 - 30 kDa. Hyaluronidase activity was detected in a band around 41 kDa in all venoms. Antigenic cross-reactivity was observed among the five venoms studied using commercial antisera produced in Brazil (AAS: AntiArachnidic Serum and PSLAS: Poly Specific *Loxosceles* Anti Serum). By ELISA there was intense cross-reactivity amongst all venoms (titre range: 64,000-512,000). By Western blotting the sera recognized mainly components of between 25-40 kDa in all venoms. Several minor components >83 kDa were also revealed. All venoms (5 μg) induced a similar local reaction in vivo, characterized by dermonecrosis, haemorrhage/palor and oedema/erythema when injected intradermally (i.d.) into the rabbit flank. However no local reaction was observed when each venom was incubated (1h, 37°C) with Brazilian commercial sera prior to i.d. injection. These results demonstrate that the venoms of the major medically important *Loxosceles* spider species have generally similar toxic and enzymatic characteristics and that the Brazilian commercial antisera are able to neutralize the effects of these venoms in a rabbit model of dermonecrotic arachnidism.

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Antivenoms - Meeting Room 3 - 1445-1500

ABSTRACT NUMBER 20401 5CR4

HYALURONIDASE INHIBITORS (SODIUM CROMOGLYCATE AND SODIUM AUROTHIOMALATE) REDUCE THE LOCAL TISSUE DAMAGE AND PROLONG THE SURVIVAL TIME OF MICE INJECTED WITH NAJA KAOUTHIA AND CALLOSELASMA RHODOSTOMA VENOMS

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Experiments have been carried out to find potent inhibitors of hyaluronidases of *Naja kaouthia* (NK) and *Calloselasma rhodostoma* (CR) venoms with the aim of reducing local tissue damage and systemic toxicities caused by the venoms. Seven drugs/chemicals known to inhibit hyaluronidases were tested for their activity on venom enzymes. These were: sodium cromoglycate (SC), sodium aurothiomalate (SAT), apigenin, kaemferol, phenylbutazone, oxyphenbutazone and fenoprofen. The results showed that SC or SAT at 10 mM, completely inhibited the enzymes of both venoms. In in vivo experiments, SC or SAT, when incubated with NK venom prior to injection, significantly reduced edema and myonecrosis. In the case of CR venom, hemorrhage, in addition to edema and myonecrosis, was also significantly reduced. In the independent type experiment, SC or SAT were effective if injected within 1 min after the injection of venom. At longer time intervals of 3 and 10 min the inhibitors were effective in reducing some parameters of local tissue necrosis but the extent of inhibition was lower. SC and SAT at 256 and 195 μg/mouse, respectively, significantly prolonged the survival time of mice receiving lethal doses of NK. In the case of CR venoms, the two inhibitors not only prolonged the survival time but also prevented death of mice receiving lethal doses of the venom. The other inhibitors were poorly soluble in water and were studied only on enzyme inhibition and prolongation of survival time; they were mostly ineffective. Thus, SC and SAT when injected immediately at the sites of bites can reduce the systemic and local toxicity of NK and CR venoms. These results suggest that administration of these drugs at the site of venom injection may be useful in reducing venom-induced local tissue damage.

Supported by a grant from the Thailand Research Fund.

Antivenoms - Meeting Room 3 - 1500-1515

ABSTRACT NUMBER 04801 5CR5

THE PRODUCTION OF CALLOSELASMA RHODOSTOMA ANTIVENOM (CR-AV) FROM EGG YOLKS OF HENS IMMUNIZED WITH VENOM AND ITS APPLICATION FOR THE TREATMENT OF SNAKEBITE PATIENTS IN VIETNAM

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In 1892, A Calmette was the first doctor in the world to successfully produce antivenom (AV) from horses. Since then, it has been saving the life of many snakebite victims. However the titer of this AV is low, side effects are relatively common and it is rather costly. The basic aim of this project solves all above problems.

Method: The hens were immunised with *Calloselasma rhodostoma* (CR) venom antigen. The eggs were collected and purified to CR IgY AV. This AV was applied to treat CR severely envenomed patients.

Result: Accomplishment of the immunization schedule in hens with CR.Ag for one year. The titer of IgY antibody is twice that in horses. An experimental IgY.AV has been produced from the egg yolks of these hens with protective effect 4 times higher and at a cost 10 times lower compared to those from horses. 30 CR snakebite patients with severe envenoming were treated with CR.IgY.AV and gave good results.

Conclusion: CR.IgY.AV was produce from immunized hens and used fro snakebite patients in Viet Nam. It has more efficacy, good safety and lower price than equine AV.

Antivenoms - Meeting Room 3 - 1515-1530

ABSTRACT NUMBER 04702 5CR6

ENVENOMING BY THE HOPLOCEPHALUS GENUS (ELAPIDAE: AUSTRALIA)

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The Australian broad headed snake genus, *Hoplocephalus* (Elapidae) contains three species, *H. bungaroides, H. bitorquatus, H. stephensii*. These three snakes are all small to medium sized and at least one species is considered endangered. Possibly because of this, they are now popular amongst herpetologists. This has resulted in a number of bites and the subsequent envenoming, with defibrination as the principle feature, has demonstrated that these snakes are capable of severe, potentially fatal envenoming. Recent experience with bites by these snakes will be discussed, indicating the extent of coagulopathy, the use of CSL Tiger Snake Antivenom in treatment, the issue of uncertain venom detection for these snakes and the diagnostic dilemma they pose.

Australian Society of Biophysics Session - Meeting Room 1&2 - 1600-1620

ASB ABSTRACT NUMBER ASB05 5DM1

DIMERIZATION MODE OF *T. MARITIMA* RIBOSOMAL STALK PROTEINS L12 IN SOLUTION

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One of the most characteristic structural features of the prokaryotic 50S ribosomal subunit is the "stalk", a protuberance that contains the protein called L7/L12. In *E. Coli*, L7/L12 is a dimeric protein which is able to undergo rapid subunit exchange (Hamman et al., 1996 *Biochemistry* **35**: 16680-6,). Recent X-ray data reported a tetrameric arrangement of the L12 proteins isolated from *Termotoga Maritima*. This structure was used to propose 2 alternative dimerization modes. In one mode, the 2 monomers of L12 form a tight symmetric and parallel dimer held together by a four helix bundle. In the other mode, the two monomers bind through their N-terminal region in an anti-parallel configuration with one monomer in a tight conformation and the other monomer adopting an elongated shape. The first mode of dimerization has been used to represent L7/L12 into the structural model of the 70S ribosome and a recent publication (Nomura et al., 2003 *Biochemistry* **42**: 4691-8) used this mode to discuss their experimental results. In this work, we present data from Förster Resonance Energy Transfer experiments using labelled cysteine mutants of *T. maritima*, which support the second mode of dimerization, i.e. in an anti-parallel orientation and an elongated configuration. We also demonstrate that the rate of subunit exchange among a population of *T. maritima* L12 is significantly slower than in the *E. coli* system.

Australian Society of Biophysics Session - Meeting Room 1&2 - 1620-1640

ASB ABSTRACT NUMBER ASB06 5DM2

INSULIN DEPENDENCE OF GLUCOSE TRANSPORTER GLUT4 IN ADIPOCYTES

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The insulin-responsive glucose transporter GLUT4 plays an essential role in blood glucose homeostasis by allowing adipose tissue and skeletal muscle to take up glucose after a meal and in the case of skeletal muscle also during exercise. To gain insight into intracellular GLUT4 trafficking, which is largely responsible for the magnitude of GLUT4-mediated glucose uptake, a novel 96 well fluorescence assay was designed based on the retroviral expression of exofacially tagged GLUT4 in 3T3-L1 adipocytes. Immunolabeling of these cells with an anti-tag antibody and a fluorescent secondary antibody respectively after fixation of the cells in the absence or presence of a cell-permeabilizing agent allows for a quantitative analysis of the amount of GLUT4 at the cell surface during insulin stimulation. This enables us to track the transition of the system from a steady state in the absence of insulin to a steady state with both an excess of external insulin and intermediate levels. A model of the kinetics involved in this transition was developed and a time constant for the transition determined as a function of applied insulin. Moreover, incubation of live cells with the anti-tag antibody in the absence or presence of insulin, followed by post-fixation immunolabeling with the fluorescent secondary antibody in the presence of the cell-permeabilizing agent demonstrates the kinetics of GLUT4 trafficking via the plasma membrane and the percentage of total intracellular GLUT4 that is involved in this process. It was found that a significant amount of GLUT4 is excluded from traversing the plasma membrane, suggesting the existence of a static insulin-insensitive GLUT4 pool (silent pool). Modelling this process we were able to estimate both the internalization and exocytosis rate constants for the GLUT4 expression at the plasma membrane, and found that whilst the internalization rate constant remains largely unaffected by insulin, the exocytosis rate constant is altered. Significantly we found that the total amount of GLUT4 involved in the cycling process in steady state changed with changing insulin levels. We will discuss the possible mechanisms for these effects.

Australian Society of Biophysics Session - Meeting Room 1&2 - 1640-1700

ASB ABSTRACT NUMBER ASB07 5DM3

CONFORMATION OF PRION PROTEIN REPEAT PEPTIDES PROBED BY FRET MEASUREMENTS AND MD SIMULATIONS

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Low complexity sequence in proteins is now recognized to be a significant proportion of coding sequence (~10%), but as such regions are generally conformationally disordered and methods to study them are not well established, their structure and functions have been little studied. As high-resolution (x-ray, NMR) methods are usually inapplicable, lower resolution methods have been used to probe the presence of both local H-bonded structure, e.g. strands and helices, as well as longer-range conformational structure. Fluorescence resonance energy transfer (FRET) is one of the latter methods.

We report the combined use of steady-state FRET experiments and Molecular Dynamics (MD) simulations to investigate conformational distributions of the prion protein (PrP) repeat system. FRET was used for the first time to probe the distance, as a function of temperature and pH, between a donor Trp residue and an acceptor dansyl group attached to the N-terminus in seven model peptides containing one to three repeats of the second decarepeat of PrP from marsupial possum $(PHPGGSNWGQ)_nG$, and one and two human PrP consensus octarepeats $(PHGGGWGQ)_nG$. In multirepeat peptides, single-Trp mutants were made by replacing other Trp(s) with Phe. As previous work has shown PrP repeats do not adopt a single preferred stable conformation, the FRET values are averages reflecting heterogeneity in the donor-acceptor distances. The T-dependence of the conformational distributions, and derived average dansyl-Trp distances, were obtained directly from MD simulation of the marsupial dansyl-PHPGGSNWGQG peptide.

The results show excellent agreement between the FRET and MD T-dependent distances, and demonstrate the remarkable sensitivity and reproducibility of the FRET method in this first-time use for a set of disordered peptides. Based on the results, we propose a model involving cation-π or π-π His-Trp interactions to explain the T- (5-85°C) and pH- (6.0, 7.2) dependencies on distance, with HW i, i+4 or WH i, i+4 separations in sequence being more stable than HW i, i+6 or WH i, i+6 separations. The model has peptides adopting loosely folded conformations, with dansyl-Trp distances very much less than estimates for fully extended conformations, for example ~16 *v.* 33, ~21 *v.* 69, and ~22 *v.* 106 Å for 1 to 3 decarepeats, and ~14 *v.* 25 and ~19 *v.* 54 Å for 1 and 2 octarepeats, respectively. The study demonstrates the usefulness of combining FRET with MD, a combination reported only once previously. In particular, initial "mapping" of the conformational distribution of flexible peptides by simulation can assist in designing and interpreting FRET experiments.

Australian Society of Biophysics Session - Meeting Room 1&2 - 1700-1720

ASB ABSTRACT NUMBER ASB08 5DM4

FLUORESCENCE PHOTOBLEACHING TECHNIQUES ON THE CONFOCAL MICROSCOPE FOR THE EXAMINATION OF PROTEIN AND CELLULAR DYNAMICS

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Fluorescence recovery after photobleaching has traditionally been employed to measure the diffusion coefficients and mobile fractions of macromolecules in cells. The emergence of GFP transfection technology and the wide availability of the confocal microscope have extended the applicability of this technique. In particular, the flexibility of design of the modern confocal microscope permits the execution of various photobleaching protocols for the examination of cellular and protein dynamics in live cells. We have used such approaches to examine protein and cellular dynamics during the erythrocytic life cycle of the malaria parasite. The design of photobleaching experiments and the information that can be gathered will be discussed

Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 01301 5PF.4

APAMIN: FUNCTIONAL EFFECTS OF ENGINEERED DESTABILISING CONFORMATIONS.

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Apamin, a neurotoxin from the venom of the European honeybee, *Apis mellifera*, binds selectively and with high specificity to small conductance, calcium-activated potassium channels (SK channels). The toxin is a conformationally stable octadecapeptide, highly resistant to physiochemical changes, chemical modification of side chains, and digestion by proteases. The peptide has two disulphide-linked bridges and consists of an α-helical core and β-turn regions; the N-terminal Asn- stabilized type 1 β-turn (involving Asn2, Cys3, Lys4 and Ala5) is connected to the C-terminal α-helix via the two disulphide bonds (Cys1-Cys11 and Cys3-Cys15). Dempsey et al (2000) have shown that an Asn2 to Ala2 substitution does not retain this N-terminal b-turn conformation, destabilising the C-terminal helix region, and hence the whole peptide. Oxidative folding of this mutant gives rise to both native and non-wild type (Cys3-Cys11, Cys1-Cys15) disulphide pairings. The present study looks at the functional effects of this amino acid substitution, and of the non-native disulphide bond pairing.

Binding studies show that the Ala2 substituted peptide has a similar affinity for apamin binding sites as the Asn2 synthetic peptide. This suggests that the N-terminal β-turn is not critical for this aspect of the toxin's activity. Suprisingly, the molecule containing mispaired disulphide bonds showed only a 30-fold reduction in its ability to displace apamin from SK channels.

Dempsey CE et al (2000) Biochemistry 39: 15944-52.

Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 06702 5PF.4

ENZYME-LIKE COMPONENTS OF SPIDER VENOMS

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cDNA libraries derived from spider venom glands of *Agelena orientalis, Chiracantium punctorium, Misumena vatia* and *Tibellus oblongus* were constructed and sequenced. The EST database of putative protein sequences was analyzed by the BLAST algorithm. Analysis of this database revealed sequences with evident homology to several types of enzyme. These putative enzymes were grouped based on their possible function. A group of serine-like proteases apparently involved in posttranslational modifications of venom proteins was selected for further analysis. The group revealed homologies ranging from 30%-94% to known serine proteases from flies, crabs, spiders and other arthropods. The highest homology was detected for a deduced sequence from *Agelena orientalis* venom. This sequence has 94% homology to the heavy chain of a peptide isomerase from *Agelenopsis aperta* that was shown to invert the L-Ser to D-Ser in Aga-TK toxin (Shikata,Y. et. all JBC v.270, 16719-16723, 1995). The gene organization of the putative peptide isomerase was defined, consisting of a light chain region followed by a heavy chain region. Furthermore, a group of trypsin-like proteases with a molecular mass of about 25 kDa was also identified in all spider venoms analyzed. Such proteases are presumably involved in toxin maturation in spider venoms.

Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 10301 5PF.4

PARALYTIC TOXINS OF THREE GASTROPOD OLIVIDAE SPECIES IN TAIWAN

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The toxins in the gastropods *Oliva miniacea, O. mustelina* and *O. nirasei* implicated in a food paralytic poisoning incident in South Taiwan in February 2002 were studied. The remaining gastropods were divided into digestive gland and edible portion and determined for toxicity by using tetrodotoxin (TTX) bioassay. It was found that the three species of gastropods contained moderate amounts of toxin in edible portion only, and the highest toxicity score was 18 MU/g for *O. miniacea*, 10 MU/g for *O. mustelina*, and 27 MU/g for *O. nirasei*. The highest average value of monthly toxicity was 40 MU/g for *O. miniacea*, 21 MU/g for *O. mustelina*, and 17 MU/g for *O. nirasei*. The toxin was partially purified from the toxic specimens of each species by ultrafiltration using a YM-1 membrane, followed by chromatography on Bio-Gel P-2 column. Analyses by high performance liquid chromatography, gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry showed that the toxin from *O. miniacea, O. nirasei* and *O. mustelina* contained TTX, and related compounds 4-epi TTX and anhydro-TTX. The paralytic shellfish poisons were not found. Hence, the causative agents in these gastropod species implicated in this food poisoning incident were identified as TTX and related substances. These three gastropod species are new toxic gastropods.

Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 12101 5PF.4

CHARACTERIZATION AND CDNA CLONING OF THE LETHAL TOXIN FROM THE VENOM OF BUNGARUS FLAVICEPS

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The major lethal toxin was purified from the venom of red-headed krait, *Bungarus flaviceps* (O. Khow et al., Toxicon 40, 463, 2002). In the present study, the amino acid sequences were determined by peptide sequencing and cDNA cloning. The toxin consisted of two polypeptide chains with a molecular weight of 13 kDa and 8 kDa under reduced condition. Amino acid sequencing of N-terminus and enzymatically digested peptides revealed that the heavy chain and the light chain were highly homologous to the A chain and the B chain of beta-bungarotoxin (beta -Bgt) from Bungarus multicinctus, respectively. The purified lethal toxin exhibited phospholipase A activity using a synthetic phospholipid as a substrate. We isolated cDNA clones encoding the heavy chain and the light chain from *B. flaviceps* venom gland cDNA library using probes based on the cDNA sequence of beta-Bgt. cDNAs of two variants of the heavy chain and one variant of the light chain were cloned. Each precursor of the two heavy chains consists of 146 amino acid residues including a signal peptide of 27 residues and a mature peptide of 119 residues. Each of them comprises 15 half-cystine residues while the A chain of beta-Bgt comprises 13 residues. The light chain is composed of 83 amino acid residues including a signal peptide of 24 residues and a mature peptide of 59 residues and comprises 7 half-cystine residues like the B chain of beta-Bgt. In conclusion, these results suggest that the lethal toxin from *B. flaviceps* is a novel isoform of beta-Bgt.

Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 12901 5PF.4

MOLECULAR EVOLUTION OF α-NEUROTOXIN GENES EXPRESSED IN THE VENOM GLANDS OF ELAPIDAE SNAKES

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The genes encoding erabutoxin (short chain neurotoxin) isoforms (Ea Eb and Ec), LsIII (long chain neurotoxin) and a novel long chain neurotoxin were cloned from *L. semifasciata* genomic library. Short and long chain neurotoxin genes were also cloned from the genom of *L. laticaudata*, closely related species of *L. semifasciata* by PCR cloning procedure. Matrix associated region (MAR)-like sequence was found in the intron I of LsIII gene. Comparative analysis of structurally relevant 11 snake toxin genes (three-finger structure toxins) revealed the process of molecular evolution of these toxins. Three-finger structure toxin genes diverged from a common ancestor through two types of evolutionary pathways (long type and short type), early in the course of evolution. And in the later state of them in each, accumulation of the mutations in exons, especially exon II, by accelerate evolution may cause the more diversification in functions. It was also revealed that MAR-like sequence found in LsIII gene was integrated in the gene after the divergence of species.

Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 14801 5PF.4

STRUCTURAL STUDY OF PEPTIDES FROM THE VENOMS OF TWO MARINE SNAILS BELONGING TO THE FAMILY TURRIDAE

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Cones, turrids and terebrids (superfamily Conacea) use venoms to capture prey, to defend from predators, and to deter competitors. The venom from each cone snail species comprises 50 to 200 distinct peptides, named conotoxins (CTX), of average size close to 30 residues, and high conformational stability. CTX bind to ion channels, receptor and transporters in a highly specific way, which has permitted to use some of them as molecular probes to study their molecular targets, and as models to design pharmaceuticals1.

Recently, we showed that the venoms of the turrids *Gemmula periscelida* and *Polystira albida* contain a large number of peptides, and determined the partial amino acid sequence of one peptide from each species. Both peptides contain a high number of Met, Arg and Tyr residues and very few, if any, Cys residues, which contrasts with all CTX. The molecular mass of the peptide from *P. albida* (11,867 Da) is clearly higher than that of any CTX.

In the present work, we conducted in a preliminary way the theoretical structural characterization of the peptides from *G. periscelida* and *P. albida*. The peptides were found to be homologous to each other and both differ structurally (probable coiled-coil motif) from all the know classes of CTX. Given that the peptide from *P. albida* was shown to be toxic to *D. melanogaster* it is possible that the one from *G. periscelida* is also toxic.

In conclusion, at least some toxins from the turrid snails may be conformationally stabilized and/or act by a mechanism distinct of those described for CTX.

1Olivera B.M. and Cruz L.J. Toxicon 39: 7-14 (2001)

Project supported by PAPIIT-DGAPA-UNAM ES 206701 and CONACYT Z-005

Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 15601 5PF.4

CLONING AND CHARACTERIZATION OF GENE ENCODING THE PRECURSOR OF BRADYKININ-POTENTIATING PEPTIDES AND C-TYPE NATRIURETIC PEPTIDE FROM AGKISTRODON BLOMHOFFI.

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Bradykinin-potentiating peptides (BPP), also known as angiotensin-converting enzyme inhibitors, repress the degradation of bradykinin and also inhibit the activity of the angiotensin-converting enzyme (ACE), which, as a whole, results in the lowering of blood pressure. C-type natriuretic peptide (CNP), a member of the natriuretic peptide hormone family, is known to be produced in both the central nervous system and vascular endothelial cells, and functions via a paracrine/autocrine transmission pathway rather than by circulation. We have previously reported the complete sequences of cDNA encoding the precursor of BPP and CNP from the venom glands of Brazilian snake, *Bothrops jararaca* and Japanese snake, *Agkistrodon blomhoffi*, and elucidated that multiple BPPs and CNP were synthesized as a single precursor [1, 2]. We screened an *A. blomhoffi* genomic library using two specific probes, which contain cDNA encoding BPPs and CNP, respectively. We isolated a genomic clone containing the gene coding for the precursor of BPPs and CNP. Genomic characterization of the BPP-CNP gene identified three exons extending over seven kilo-base pairs of genomic DNA. Each exon contains 5' untranslated region, signal sequence and BPP-coding region, spacer and CNP-coding region, and 3' untranslated region, respectively.

[1] Murayama, N. et al. (1997) Proc. Natl. Acad. Sci. USA. 94, 1189-1193.
[2] Murayama, N. et al. (2000) Eur. J. Biochem. 267, 4075-4080.

Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 15901 5PF.4

ISOLATION AND BIOCHEMICAL CHARACTERIZATION OF PEPTIDES FROM THE AUGER SNAIL TEREBRA SUBULATA

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The family Terebridae is one of the four major groups of venomous marine gastropods in the in the superfamily Conacea. We characterized three peptide components from the venom of the species *Terebra subulata*. These are 40-41 amino acids in length with 3-4 disulphide linkages, similar to conotoxin superfamilies. Injection of one of the purified peptides produced uncoordinated movements in the nematode *C. elegans*. Moleceular cloning failed to show the highly conserved sequence features found in conopeptide gene superfamilies with similar arrangement of cysteine residues. Furthermore, *Terebra* may have evolved larger venom components that are less highly post translationally modified.

Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 16104 5PF.4

ALPHA-CONOTOXIN MI INTERACTION WITH TORPEDO CALIFORNICA NICOTINIC ACETYLCHOLINE RECEPTOR. MOLECULAR MODELLING STUDIES.

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To date a number of 3D structures of transmembrane proteins has been solved but this is not the case for neurotransmitter-gated ion channels. One of the members of this family is the nicotinic acetylcholine receptor (nAChR). Several diseases are related to disfunction of nAChR, thus stimulating its biochemical characterization. 3D structure of acetylcholine-binding protein from *Lymnaea stagnalis*, that is homologue of nAChR, has been solved recently. We used it to model spatial structure of extracellular part of nAChR from *Torpedo californica*. The model was tested via 3D-profile method. The profile obtained for the constructed 3D model was able to recognize its own amino acid sequence in the Swiss-Prot database. This provides strong grounds for validity of the model. The model was employed to study binding of alpha-conotoxin MI, which is one of the most studied ligands to this receptor.

It has been shown that the residues Pro6, Ala7, Gly9, and Tyr12 of alpha-conotoxin MI contribute to its high affinity binding to nAChR [Bren N., Sine S.M., (2000) JBC 275, 12692-12700]. Tyr12 has the most profound impact. To examine if this effect is mediated by the changes in structure and/or dynamics caused by the replacement of Tyr12, the wild toxin and its Y12T mutant were studied via NMR spectroscopy and molecular dynamics simulation. It was shown that spatial structure and dynamical behavior of the mutant are very similar to those of the wild type toxin. A conclusion was reached that Tyr12 directly interacts with the receptor.

Docking of the alpha-conotoxin MI to the model of the extra cellular part of the nAChR was studied using the programs GRAMM and GOLD. In the first case (rough geometrical docking) two putative binding sites were delineated. They lie on the interface between alpha/delta and alpha/gamma subunits of nAChR. Subsequent refinement of the position of alpha-conotoxin in the binding site was done with the program GOLD. Among the best 10 structures of receptor-toxin complexes there is one that is consistent with the data obtained biochemically. The proposed approach is currently being used to characterize in detail intermolecular interactions in the active site of the receptor.

Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 16401 5PF.4

SYSTEMATIZATION OF SNAKE VENOM NEUROTOXINS BASED ON STRUCTURE AND FUNCTION

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Snake neurotoxins are defined as toxins that act presynaptically or postsynaptically at the neuromuscular junction. They display diverse pharmacological properties and are attractive candidates for the development of drug targets and therapeutics. A growing number of snake neurotoxins in the public databases have created a need for an improved data management. In our work, bioinformatics-based approach was employed to build a specialised database for snake neurotoxins. This database contains 293 unique sequences that are well annotated. This data was further classified based on thier molecular targets to determine structure-function relationships. This single and organised databse provides a platform for the analysis of unknown or novel neurotoxin cDNAs. We envisage that this database can serve as an additional tool for proper, accurate and afficient data analysis of snake neurotoxin proteins and cDNAs.

Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 16901 5PF.4

COMPARISON OF THE PLATELET AGGREGATION INDUCED BY ACUTOBIN,THROMBIN AND ADP

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The effects of acutobin,a thrombin-like enzyme purified from *Deinagkistrodon acutus* venom with molecular weight about 26 kd ,thrombin and ADP on platelet aggregation were compared and the effect of acutobin on the action of the latters were studied in rabbits. 10 ml blood were drawn by heart puncture from rabbits and mixed with 1.1 % EDTA-Na2 (9:1),then washed with Tyrode's(Ca^{2+} free) solution and suspend in Ca^{2+}-Tyrode's solution to prepare platelet suspension. Different concentraction of acutobin ,thrombin and ADP were added to 200 μl platelet suspension/tube 37°C preincubated for 5 min respectively. The aggregation rate was recorded with aggregometer. The results showed that acutobin at concentracttion 0.4~1.0 U/50 μl induced platelet aggregation by 7~30 % with positive correlation. The aggregation rate (60%) induced by thrombin (0.5 U/50 μl) was decreased to 30-50% by acutobin ,but the aggregation rate(20%) induced by ADP (20μM/50 μl) was not significantly affected by acutobin if platelet was preincubated with acutobin. These results suggested that acutobin can aggregate washed platelet but inhibit aggregation induced by thrombin and not affected the aggregation induced by ADP.

Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 18001 5PF.4

ISOLATION AND CHARACTERIZATION OF TIGRINOBIN, A SERINE PROTEASE WITH THROMBIN-LIKE ACTIVITY, FROM THE VENOM OF THE TIGER RATTLESNAKE (CROTALUS TIGRIS)

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Thrombin-like serine proteases are common among viperid snake venoms, and poorly known venoms have the potential to yield new structural or new substrate specificity variants of these enzymes. We initiated a study of venom from the tiger rattlesnake (*Crotalus tigris*), a species with limited distribution in the Sonoran desert of the North American southwest which produces a venom with high toxicity and low metalloprotease activity. In addition to a potent homolog of crotoxin and Mojave toxin, several serine proteases with different substrate specificities are present. We isolated a thrombin-like protease from crude *C. tigris* venom using a single step RP-HPLC method. MALDI-TOF mass spectrometry indicated a mass of 29.1 kD for this protease, which was termed tigrinobin. Relative to human thrombin, tigrinobin greatly accelerated prothrombin time of freshly collected citrated human plasma. Tigrinobin also promoted clot formation in buffered fibrinogen clotting assays. Tigrinobin shows 100% N-terminal sequence homology (first 18 residues) with venombin A from *Lachesis muta* and several other viperid venoms, but its sequence is quite different from bovine or human thrombin. To compare fibrinogen clotting activity by human thrombin and tigrinobin, fibrinogen and enzyme were incubated at 37 °C for 5 minutes; the clot formed was removed and unreacted fibrinogen was precipitated via addition of chilled 10% TCA followed by centrifugation. Supernatant was analyzed by MALDI-TOF-MS and fibrinopeptides A (1536.7 D) and B (1569.7) were identified in spectra of thrombin-catalyzed digests. Tigrinobin catalyzed the release of fibrinopeptide A (observed mass = 1536.9 D), but fibrinopeptide B was not released; however, a second peptide (4571 D) was observed. Time course assays indicated that tigrinobin rapidly catalyzed the release of fibrinopeptide A, and the second peptide was more slowly hydrolyzed. Like other venom thrombin-like proteases, tigrinobin shows superficial similarities to thrombin, but clot-forming activity appears to have significant differences from thrombin. Release of a large peptide fragment from the Bbeta subunit of fibrinogen may be a characteristic activity of venom thrombin-like enzymes and may be related to the rapid onset of fibrinogenopenia with absence of patent clots commonly seen upon human envenomation by many viperid snakes.

Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 18201 5PF.4

THE SYNTHETIC INHIBITION OF ECHIS OCELLATUS VENOM METALLOPROTEINASES

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Objective: Envenoming by the carpet viper *Echis ocellatus* in savannah Nigeria is a major concern. At some times of the year, victims can occupy 74% of regional hospital beds. The primary symptoms of *E ocellatus* envenoming are local and systemic haemorrhage. Venom-specific antivenom is inadequate at alleviating local haemorrhagic and necrotic pathology unless administered soon after the bite. At present, there is little/no antivenom in Africa. The objective of this study is to determine whether inhibitors of mammalian metalloproteinases (MMPs) will serve to inhibit the pathological activity of E ocellatus' and other venom metalloproteinases (SVMPs). Rationale: *Echis* venoms contain potent zinc SVMPs (reprolysins) that are primarily responsible for the haemorrhagic pathology characteristic of viperine envenoming. Structural homologs of SVMPs have been identified in mammalian tissues (matrix metalloproteinases MMPs). MMPs are structurally and functionally similar to SVMPs, since both seem to be primarily involved in the hydrolysis of the extracellular matrix based on common structural features. The activities of MMPs have been curtailed by broad-range synthetic inhibitors such as Bay-12-9566, CGS-23027A, Marimastat and AG-3340. The high degree of tertiary structure conservation among the reprolysin and other MMP subfamilies suggests that the principles that govern the interactions of substrates and inhibitors with members of these subfamilies are likely to be similar if not identical. Methods FPLC separation of E ocellatus venom resulted in the purification of three SVMPs; EoVMP1, a non-haemorrhagic 24 kDa short-chain metalloproteinase; EoVMP2, a haemorrhagic 56 kDa long-chain metalloproteinase, and EoVMP3, a 65 kDa haemorrhagic long-chain metalloproteinase. The actions of these enzymes on components of the basal lamina and haemostatic system were assayed both in vitro and in vivo. Antibodies raised against EoVMP1 and EoVMP2, as well as commercial antivenoms, metal ion chelators and the above MMP inhibitors were assayed against numerous SVMPs and snake venoms in an attempt to neutralise their enzymatic activities. Results: Crude *E ocellatus* venom and its purified SVMPs were shown to selectively degrade several components of the basal lamina and haemostatic system thereby implicating their involvement in the pathology of envenoming. Inhibition of the activities of a range of snake venoms and their constituent reprolysins was achieved to varying degrees using the fore-mentioned compounds. Conclusions: This information suggests that it may be possible to evolve a new strategy of snake-bite management in parallel (or as an alternative) to antivenom. Specific venom components within a wide range of viper venoms may be inhibited by broad-spectrum synthetic peptides.

Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 18302 5PF.4

BOTHROPS INSULARIS VENOM PREDICTIVE PROTEOME

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Bothrops insularis is an endemic snake found in Ilha da Queimada Grande, 20 miles from the Brazilian coast. Differently from other bothropic snakes it preys on birds and some invertebrates available in its habitat. Since snake venoms contain a complex mixture of proteins (enzymes, biologically active peptides) and non-proteic components it is thought that evolutionary pressure could have resulted in an adaptation of its venom components providing divergent toxins to better capture the preys. A group from Instituto Butantan, headed by Dr. Paulo L. Ho is performing a survey of gene expression and diversity in *B. insularis* venom glands through the generation of expressed sequence tags (ESTs) and putting out a wealth of genomic data. Thus, *B. insularis* venom (donated by Instituto Butantan) seems to be a good model system in order to study toxin expression. The elucidation of the proteome follows the use of preparative 2D-PAGE to separate proteins (ranging from 100 to 10 KDa) in two different conditions: using DTT in the first and second dimension and using DTT only in the second dimension. Different reproducible patterns were observed in both conditions even when home-made gels from different laboratories were compared. Major spots were used to generate tryptic hydrolysates for peptide mapping by MALDI-ToF-MS. Searches in Gene/ ESTs data banks allowed identification and a possible function assignment for the proteins. This is the first joint effort in Brasil to identify proteins by a proteomic network.

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Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 19001 5PF.4

PSP-TOXIFICATION OF THE CARNIVOROUS GASTROPOD RAPANA VENOSA AND SOME SHELLFISH INHABITING THE ESTUARY OF NIKOH RIVER, HIROSHIMA BAY, HIROSHIMA PREFECTURE, JAPAN

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Hiroshima Bay is one of the largest culturing fields of oyster in Japan where the paralytic shellfish poison (PSP) infestation of bivalves such as cultured oyster have been occurred since 1992. During surveillance on the toxicity of invertebrates such as bivalves inhabiting the estuary of Nikoh river, Hiroshima Bay, some mollusks including carnivorous gastropods rapa whelk *Rapana venosa* and a snail *Omphalius rustuicus*, were found to contain toxins which showed paralytic actions on mice. The maximum toxicity of *R. venosa* viscera was 11.4MU/g and the edible part of *O. rusticus* was 3.8MU/g, respectively. The toxic principle was identified as PSP toxins such as gonyautoxin 2 (GTX2) and hyneosaxitoxin (hyneoSTX) by HPLC. The toxin compositions of causative phytoplankton *Alexandrium tamarense* and some feed bivalves inhabiting the same site, were also determined. From the toxin patterns of these organisms, mechanism for their toxicification can be assumed that PSP toxins produced by phytoplankton such as *A.tamarense*, is transferred to and accumulated in plankton feeder bivalve short-necked clam, and then moved to carnivorous organisms *R .venosa* through predation.

Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 19601 5PF.4

UNIQUE ALPHA-FIBRINOGENASE FROM VIPERA LEBETINA VENOM – CLONING AND EXPRESSION.

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Snake venoms contain serine and metalloproteinases that affect the hemostatic system by a variety of mechanisms. Specific activators of blood components (protein C, factor V, plasminogen) were found among serine proteinases. Snake venom serine proteinases cleaving fibrinogen may be divided into coagulants (thrombin-like enzymes) which split off the fibrinopeptides A and/or B, and anticoagulants that preferentially degrade fibrinogen beta chain into fragments that are not clottable by thrombin. Central Asian *Vipera lebetina* venom contains various serine proteinases: factor V activating enzyme, kininogenases, and two anticoagulant serine proteinases, beta-fibrinogenase and alpha-fibrinogenase (VLAF) with unique specificity. Alpha-fibrinogenase degrades alpha chain of fibrinogen, prolonging its clotting time by thrombin. VLAF is inactive towards low molecular weight esters of arginine, lysine and tyrosine. VLAF is inhibited by serine proteinase inhibitors PMSF and DFP, and its N-terminal sequence is homologous to snake venom serine esterases. Cloning and sequencing of the cDNA encoding VLAF revealed some peculiarities in its primary structure: 1) from the three Asp residues characteristic to trypsin-type enzymes, Asp189 necessary for binding of the basic residue of the substrate was replaced by Gly189, and 2) in the close proximity of the substrate-binding cleft, the sequence of three arginines (Arg185-Arg187) may cause steric and electrostatic hindrance for the positively charged Arg-containing substrates (Siigur et al. 2003).

Production of the recombinant enzyme appeared to be a serious problem. It cannot be expressed as a mature protein alone but needs expression as a fusion protein. The fusion with maltose binding protein (MBP) was used in E. coli BL21(DE3) strain. This system allows production of a soluble protein facilitating the purification and activation of the enzyme. Amylose agarose was used as affinity support for fusion protein purification. The recognition sequence for factor Xa was inserted between MBP and VLAF coding regions and VLAF was separated from MBP after site-specific cleavage by factor Xa. The released VLAF was not enzymatically active.

Ref.: Siigur et al. Thromb.Haemost. 89,826 (2003)

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Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 22601 5PF.4

DEGREE OF SPECIALISATION IN VENOM-PRODUCING CELLS OF PSEUDONAJA TEXTILIS

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The Australian common brown snake, *Pseudonaja textilis*, possesses one of the most potent venoms so far isolated from land snakes. Considerable effort has gone towards identifying and characterizing the components of the venom but the mechanism of its synthesis is not fully understood. It is not clear for instance whether individual cells are responsible for production of the composite venom or if specific cells are responsible for production of different venom components.

In this study, we examined the synthesis of three different venom components: textilotoxin (a pre-synaptic neurotoxin), prothrombin activator, and pseudonajatoxin b (a post-synaptic neurotoxin). Freshly collected, paraformaldehyde-fixed tissue was divided and processed into paraffin for immunohistochemical staining at light microscopic (LM) level and LR white for electron microscopic (EM) immunogold studies. In each approach a double immunostaining procedure was used allowing different combinations of antibodies to be examined concurrently on the same tissue section. These studies found that all venom-producing epithelial cells were immunoreactive for each of the venom components examined, although the intensity of immunostaining was variable for each antibody. Immunohistochemical staining was detected by LM along the apical cytoplasm of epithelial cells. Immunogold labeling revealed a similar pattern with the highest distribution of gold particles over secretory vacuoles. Although at lower concentration gold particles were also associated with rough endoplasmic reticulum and Golgi complexes. It is not clear whether the variation in intensity of staining for the different components examined is due to the concentration of antigen (amount present) or is a function of the range of epitopes against which antibody is directed and/or the affinity of the antibody. Further studies will address these questions. Nevertheless, these results demonstrate that each venom-producing cell of the Australian common brown snake is capable of synthesizing the venom components, textilotoxin, prothrombin activator and pseudonajatoxin b.

Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 24502 5PF.4

ISOLATION AND CHARACTERISATION OF A CONE SNAIL PROTEASE WITH HOMOLOGY TO CRISP PROTEINS OF THE PATHOGENESIS-RELATED PROTEIN SUPERFAMILY

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Here we report the characterisation, cloning and structural homology modelling of Tex31 from the venom duct of *Conus textile*. Tex31 was isolated to > 95% purity by activity-guided fractionation using a p-nitroanilide substrate based on the putative cleavage-site residues found in the propeptide precursor of conotoxin TxVIA. Tex31 requires four residues, including a leucine N-terminal of the cleavage site, for efficient substrate processing. The sequence of Tex31 was determined using two degenerate PCR primers designed from N-terminal and tryptic digest Edman sequences. A BLAST search revealed that Tex31 was a member of the PR protein superfamily and most closely related to the CRISP family of mammalian proteins which possess a cysteine-rich C-terminal tail. The pathogenesis-related (PR) protein superfamily is widely distributed in the animal, plant and fungal kingdoms, and is implicated in human brain tumour growth and plant pathogenesis. The precise biological activity of PR proteins, however, has remained exclusive. An homology model constructed from two PR proteins revealed that the likely catalytic residues in Tex31 fall within a structurally conserved domain found in PR proteins. Thus, it is possible that other PR proteins may also be substrate-specific proteases. To confirm the catalytic residues involved, a structural analysis of Tex31 will be undertaken. As only microgram quantities of native Tex31 can be obtained, we have attempted to express recombinant Tex31 in bacteria and yeast. Sequence analysis of PCR products, amplified using gene specific primers, have identified proteins with sequence homology to Tex31 in a number of other *Conus* species.

Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 24503 5PF.4

ALLOSTERIC A1-ADRENOCEPTOR ANTAGONISM BY THE CONOPEPTIDE ρ-TIA

Iain A. Sharpe†§, Linda Thomas†, Marion Loughnan†. Leonid Motin§, Elka Palant¶, Daniel E. Croker¶, Dianne Alewood¶, Songhai Chen‡, Robert M. Graham‡, Paul F. Alewood†¶ and Richard J. Lewis†§¶

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Cone snails use venom containing a cocktail of peptides known as conopeptides or conotoxins to capture their prey. Many of these peptides target mammalian receptors, often with exquisite selectivity. A peptide, called ρ-TIA, contained in the venom of the predatory marine snail *Conus tulipa*, has previously been shown to act as a reversible non-competitive antagonist at the α_1-adrenoceptor1. Here, we further characterize the pharmacological activity of ρ-TIA as well as investigate structure-activity relationships. In the isolated rat vas deferens, ρ-TIA inhibited a1-adrenoceptor-mediated increases in cytosolic Ca^{2+} concentration that were triggered by norepinephrine, but did not affect presynaptic α_2-adrenoceptor-mediated responses. In radioligand binding assays using [125I]-HEAT, ρ-TIA displayed slightly greater potency at the α_{1B} than at the α_{1A} or α_{1D} subtypes. Moreover, although it did not affect the rate of association for [3H]-prazosin binding to the α_{1B}-adrenoceptor, the dissociation rate was increased, indicating non-competitive antagonism by ρ-TIA. N-terminally truncated analogs of ρ-TIA were less active than the full-length peptide, with a large decline in activity observed upon removal of the fourth residue (Arg4). An alanine scan of ρ-TIA confirmed the importance of Arg4 for activity, and revealed a number of other residues clustered around Arg4 that contribute to the potency of ρ-TIA. The unique allosteric antagonism of ρ-TIA, resulting from the interaction with receptor residues that constitute a binding site distinct from that of classical competitive α_1-adrenoceptor antagonists, may allow the development of inhibitors that are highly subtype selective.

1Sharpe, I.A., Gehrmann, J., Loughnan, M.L., Thomas, L., Adams, D.A., Atkins, A., Palant, E., Craik, D.J., Adams, D.J., Alewood, P.F. and Lewis, R.J. Nature Neuroscience 4(9), 902-907 (2001).

Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 17202 5PF.4

NEUROPROTECTIVE ACTIVITY OF A NOVEL PEPTIDE DERIVED FROM THE AMINO ACID SEQUENCE OF THE PHOSPHOLIPASE A2 INHIBITOR FROM PYTHON RETICULATUS SERUM

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From the primary structure of Python reticulatus serum protein termed "Phospholipase A2 Inhibitor from Python (PIP) ", we have identified a peptide sequence that can strongly inhibit phospholipase A2 (PLA2) activity. The 17-residue synthetic peptide P-NT.II is able to inhibit the catalytic activity of the snake venom sPLA2 (crotoxin B) as well as that of the crude and purified synovial secretory(s) PLA2 of patients with arthritis. P-NT.II inhibits sPLA2 as effectively as 12-epi-scalaradial, a selective inhibitor of sPLA2, with a 5-fold selectivity over cytosolic (c) PLA2 (IC50 4.9 vs 23.9 μM). Using this newly identified sPLA2-selective inhibitor P-NT.II, we were able to examine the relative contributions of sPLA2 and cPLA2 in the cytosolic rat brain fractions before and after intracerebroventricular injections of a neurotoxin kainite. Our findings indicate that sPLA2 is as important as cPLA2 in mediating ischemic and oxidative injuries in the rat brain. The level of sPLA2 increases further in the hippocampus after kainate induced excitotoxic injury. P-NT.II also exhibits high anti-neurotoxic activity when used in organotypic hippocampal slice cultures. Addition of P-NT.II (6 μM), or 12-epi-sclaradial (20 μM) as a positive control, to cultured slices before kainate application prevents the decrease in GluR 1 immunoreactivity, indicating protective capacity of the peptide to kainate-induced neuronal injury. The experimental evidence for the direct and specific binding of the active biotinylated-P-NT.II to sPLA2, and a relatively weaker binding to cPLA2 was demonstrated by ELISA. The overall findings provide evidence that group IV cPLA2 as well as group II sPLA2 have a unique role in kainate-induced neurotoxicity, and highlight the important role of sPLA2 in excitotoxic brain injury. Attempts have also been made to generate inhibitors with improved potency using an analogue screen of P-NT.II.

Poster Session 5PF.4 - Glass Foya - 1100-1140

ABSTRACT NUMBER 5PF.4

GENERATION OF DIMERIC SCFV ANTIBODY FRAGMENTS THAT NEUTRALIZE THE WHOLE VENOM FROM THE SCORPION *CENTRUROIDES NOXIUS* HOFFMAN.

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** Correspondence*

The high morbidity and mortality from scorpion stings in Mexico is a serious health problem. Equine $F(ab)_2$ antivenom is used as part of the treatment for scorpion stings in Mexico. This last generation of scorpion-sting antidotes is safer and more efficient than earlier preparations. Nevertheless, a recombinant antidote that consists exclusively of relevant anti-venom antibodies is the ideal option for a new generation of improved antivenoms.

There are many advantages of using a dimeric scFv instead of intact IgGs or $F(ab)_2$: (1) Their production is less expensive and more reproducible; (2) because they are specific, a lower dose of foreign protein can be inyected; (3) their small size facilitates rapid distribution and clearance, which reduces the risk of undesired immunological reactions.

In this study, we report the production of 14 dimeric scFv's by means of phage display with an immunized chicken library. They are all directed against the mammal toxin Cn2, which is the major and second most toxic component of the venom of the dangerous mexican scorpion *Centruroides noxius* Hoffman. Two of the scFv's, C21 and C30, are independently capable of neutralizing the effects of both the toxin Cn2 and the whole venom. C21 and C30 bind to Cn2 with high affinity (4 and 9 X 10^{-10}M respectively) and are very stable, which makes them very good candidates for use as recombinant anti-venom against the sting of the scorpion *Centruroides noxius*.

ASB Poster Session 5PF.4 - Glass Foya - 1100-1140

MODELLING OF HIGH-OLIGOMERS OF COLLECTINS – WHAT DETERMINES THE SPATIAL DISTRUBUTION OF THE CARBOHYDRATE RECOGNITION DOMAINS

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Collectins are soluble effector proteins that target carbohydrate structures on the surfaces of invading pathogens, and play an important role in innate immune defence. The main collectins are mannose binding lectin (MBL) in blood plasma and pulmonary surfactant protein (PSP) in lung. The collectins are assembled as oligomers (typically 6-8) of trimeric subunits, each subunit consisting of a short N-terminal tail, a triple helical collagen region, a short trimeric coiled-coil linking domain and a C-type lectin carbohydrate recognition domain (CRD). While carbohydrate binding to a single CRD is weak ($K_d \sim$ 1mM), greater avidity is obtained by binding to multiple CRDs. Formation of the trimeric subunits into higher order oligomers with a bouquet-like structure enables the repeat sugar groups on a microbial surface to be bound to a large number of different CRDs. As the microbial oligosaccharide binding interaction is cooperative over many CRDs, the 3D structure not only of the individual CRD binding-site but their arrangement into trimers and, thence, into higher order oligomers is critical — not only for effective binding but discrimination for binding host oligosaccharides.

A large number of studies have been undertaken on the mode of carbohydrate binding to an individual CRD, and numerous crystal structures are available for the CRD and neck region of the trimers. The distribution of the trimers in the higher order oligomers has, however, been studied very little, and structural models of the collagenous and N-terminal domains and their influence on the distribution of the CRDs are available only from electron micrograph images. We are addressing this deficit using a combination of sequence analysis and molecular modelling methods to extend the crystallographic and EM data and create 3-D models of oligomers. We have determined a number of factors that both confer rigidity or allow flexibility in the overall distribution of the carbohydrate binding sites in the higher-order structures. Models of the "kink" caused by the Xaa-Yaa-Gly collagen interrupt in both human MBL and SP-A have been constructed, and these compare well with the structures visualised by EM. The models also take into account post-translational modifications such as hydroxylation of Pro in the collagen repeats.

ASB Poster Session 5PF.4 - Glass Foya - 1100-1140

STRUCTURAL ANALYSIS OF MYOSIN BINDING PROTEIN – C

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Myosin binding protein-C (MyBP-C) is an important protein in striated muscle for the structure and function of the sarcomere. It is a multi-domain molecule belonging to a class of fibrous proteins that include titin and telokin. Like titin, MyBP-C is composed of immunoglobulin class I (IgI) and fibronectin type III (FnIII) motifs connected like 'beads on a string'. There are three isoforms of MyBP-C found in humans; slow-skeletal, fast-skeletal and cardiac. The cardiac form differs from the skeletal forms in three ways, firstly the cardiac isoform has an extra IgI motif at the N-terminus, secondly two extra phosphorylation sites are found between motifs one and two and finally one of the central IgI domains contains a proline/charge rich insert.

The importance of cardiac MyBP-C in the sarcomere is highlighted by the heart disease familial hypertrophic cardiomyopathy (FHC). FHC is caused by mutations in sarcomeric proteins that disrupt the structure, regulation or function of the sarcomere. More than 20 % of all FHC mutations are found in cardiac MyBP-C, some of which are associated with a poor prognosis.

Multiple alignments using different isoforms of MyBP-C from a range of species identified conserved structural regions, which are perhaps important for function. Modelling based on these alignments and the known structures of FnIII domains from titin and IgI domains from titin, telokin and twitchin, produced more information about key residues and regions in MyBP-C. Based on these findings, which will be presented, motifs one and two (including the phosphorylation sites) are currently being cloned and used for further experimentation.

ASB Poster Session 5PF.4 - Glass Foya - 1100-1140

SS-NMR STUDIES OF B-AMYLOID PEPTIDE-LIPID INTERACTIONS

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The major components of aggregated plaques in the brains of Alzhiemer's Disease (AD) patients consist of β-Amyloid peptides (Aβ). Aβ peptides are 39-42 amino acid in length and are the proteolytic products of the larger amyloid precursor protein (APP). Recent studies have shown that Aβ undergoes a structural transition to adopt a β-sheet conformation[1, 2] in plaques but there is still debate regarding the nature of the aggregated species and its relationship with the observed neurodegeneration. Lipid bilayer membranes are an important factor regulating peptide structure[3]. Therefore, understanding the structural changes in both the peptide and the lipid bilayer under a variety of conditions is an important step in understanding the relationship of peptide-membrane interactions in AD.

Presence of metals such as copper and zinc may facilitate neurodegeneration in AD[4] and such a relationship is supported by an increase in metal content in the brain of AD patients[5]. EPR studies in model membranes suggest a possible ligand interaction between the metal and histidine residues of the peptide, which could affect Aβ conformation and action in membrane bilayers and may act as an intermediate to the formation of aggregates and/or plaques[3, 6].

We will present solid-state NMR (utilising phosphorus and deuterium spectra) results of Aβ peptide-lipid interactions to study these interactions. Structural information obtained from data at different pH both in the presence and absence of cofactors such as metals (Cu, Zn) will probe the interaction of Aβ with lipid bilayers to gain insight into membrane interactions, the formation of aggregated species and possible means of inhibition.

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[3] T.-L. Lau, K.J. Barnham, C.C Curtain, C.L. Masters, F. Separovic, *Aus. J. Chem.*, **2003**, *56*, 349.
[4] A.I. Bush *Trends in Neuroscience*, **2003**, *26*, 207
[5] M.A. Lovell, J.D. Robertson, W.J. Teesdale, J.L. Campbell, W.R. Markesbery, *J. Neurological Sci.*, **1998**, *158*, 47.
[6] C.C. Curtain, F.E. Ali, Danielle G. Smith, A.I. Bush, C.L. Masters, K.J. Barnham, *J. Biol. Chem.*, **2003**, *278*, 2977.

ASB Poster Session 5PF.4 - Glass Foya - 1100-1140

STRUCTURE OF VIRGINIAMYCIN VM1 REVISITED

Jason Dang[1], Frances Separovic[2], Robert P. Metzger[3], Robert T. C. Brownlee[1].
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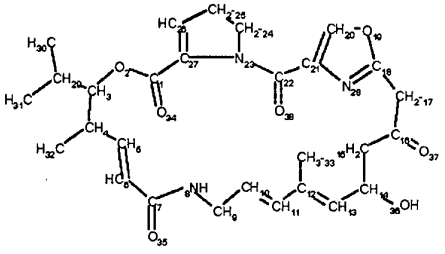
NMR spectroscopy techniques have been used in the study of three dimensional structure of Virginiamycin VM1 (an antibiotic active against Micrococcus aureus) in various solvents. The X-ray crystal structure of VM1 has previously determined in 1974 [1] but our NMR study focuses on solution structures. It was found that VM1 in CDCl3 and MeOD are similar but somewhat different to the X-Ray crystal structure. The complete assignments of VM1 have been derived from the combination of 1D and 2D NMR experiments. XEASY program was used for processing NOESY and ROESY experiments and 39 and 37 NOEs were observed for VM1 in CDCl3 and MeOD, respectively. Residue library for VM1 was created and added to DYANA library for simulated annealing. Structures were found with backbone RMSD of 0.14 +/- 0.16 and restraints violations less than 0.4Å. Further minimisation with steepest descents, conjugate gradients and dynamics using Insight/Discover software showed that the energies were comparable in a group of derived structures.



Virginiamycin VM1

Durant, F.; Evrard, G.; Declercq, J. P.; Germain, G. Namur, Belg. Crystal Structure Communications (1974), 3(3), 503-10.

ASB Poster Session 5PF.4 - Glass Foya - 1100-1140

THE EFFECT OF EQUINATOXIN II ON PHOSPHOLIPID MEMBRANES.

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* *School of Chemistry, University of Melbourne, VIC 3010*

Equinatoxin II (Eqt II) is a 20kDa cytolysin from the sea anemone, *Actinia equina*. EqtII causes lysis of phosphatidylcholine (PC) vesicles containing sphingomyelin (SM), with the greatest lysis occurring at a 1:1 PC:SM molar ratio [1]. This pore-forming toxin has been found to also affect the phase transition temperature and molecular motions of phospholipids in hydrated dispersions of PC:SM (10:1), even at a low toxin-lipid ratio (1:1100) [2].

The effect of EqtII on the phase transition temperatures of hydrated lipid dispersions with different compositions of deuterated dimyristoyl-PC (d_{54}-DMPC), SM and cholesterol (Chol), are being investigated by ^{31}P and ^{2}H solid-state (SS) NMR. Determination of the phase and order of the lipids can be obtained using ^{31}P SS-NMR, for the phospholipid-headgroups, and ^{2}H SS-NMR, for the deuterated acyl chains [2]. Spectra of d_{54}-DMPC:SM:Chol (1:1:1), d_{54}-DMPC:SM (1:1) and d_{54}-DMPC:Chol (1:1) at various temperatures have been obtained. Comparison will be made to spectra with EqtII and the effect of cholesterol on lipid domain formation with the toxin will be investigated.

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ASB Poster Session 5PF.4 - Glass Foya - 1100-1140

THE BINDING OF AN N-TERMINAL MUTANT OF COFILIN TO ACTIN

D. Chhabra, N.J. Nosworthy and Cristobal dos Remedios

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Cofilin is an essential modulator of actin in all eukaryotic cells. It regulates the state of actin assembly during cell movement and cell division, as well as having potential roles in gene expression and cell signalling. An atomic model of the cofilin-actin complex has not yet been determined, although several regions of cofilin, particularly its N-terminal region, have been implicated in its interaction with subdomains 1 and 3 of the G-actin monomer. Low resolution electron microscope studies of the cofilin-actin filament have also revealed that cofilin bridges subdomains 1 and 3 of the actin subunit above to subdomains 1 and 2 of the subunit below, consequently changing the twist and crossover length of the filament. This suggests that cofilin has at least 2 sites capable of interacting with actin (see review by dos Remedios et al., for detailed report of the cofilin-actin interaction).

Investigation of the actin-cofilin complex by fluorescence techniques have so far proved difficult due to the inability of cofilin to be labelled by fluorophores. To overcome this hurdle, we have used molecular biology techniques to add 5 residues, including 2 cysteines, to the N-terminus of cofilin, as previously described by Obinata and colleagues. This N-terminal mutant of cofilin (N-cys-cofilin) was subsequently labelled with iodocaetamide-fluorescene.

We have demonstrated that labelled N-cys-cofilin differs markedly from the native protein, displaying altered actin binding properties. We are currently investigating any structural changes using circular dichroism spectroscopy and electron microscope reconstructions.

dos Remedios, C.G., Chhabra, D., Kekic, M ., Dedova, I., Tsubakihara, M., Berry, D. and Nosworthy, N.J. (2003) Actin Binding Proteins and Regulation of Cytoskeleton Filaments. Physiological Reviews 83: 433-473.

Nagaoka, R., Kusano, K., Abe, H. and Obinata, T. (1995) Effects of cofilin on actin filamentous structures in cultured muscle cells. Journal of Cell Science 108: 581-593.

ASB Poster Session 5PF.4 - Glass Foya - 1100-1140

EPR STUDIES OF TWO FORMS OF THE S_2 STATE MULTILINE SIGNAL IN PHOTOSYSTEM II.

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The oxygen evolving complex (OEC) of photosystem (PSII) catalyses the oxidation of water to molecular oxygen. It consists of four Mn ions, one Cl and one Ca ion in an as yet undetermined geometry. The OEC is capable of storing, in a cyclic manner, four oxidizing equivalents before it oxidizes water to molecular oxygen in a concerted process and the cycle starts over. The intermediate oxidation states of the OEC are denoted $S_0 \ldots S_4$, where the suffix refers to the number of electron holes stored. The S_2 state is paramagnetic and gives rise to the Mn hyperfine 'multiline' EPR signal. Another broad, featureless EPR signal centred around g=4.1 also stems from the S_2 state. Small mono-alcohols are routinely added to the measurement buffer (~1M) as only the multiline signal is developed under those conditions.

In this study we show that the multiline signal exists in two different forms, one narrower than the other, which both stem from fully functional centres. Methanol added to the measurement buffer favours the formation of the narrower form on the first turnover of the enzyme, whereas addition of ethanol favours the broad form. However, on the second turnover of the enzyme, the broad form of the signal predominates, regardless of buffer additions. We also show that methanol binds to the active site. The structural implications of this phenomenon on the OEC are discussed.

ASB Poster Session 5PF.4 - Glass Foya - 1100-1140

COMPARATIVE MODELLING OF PTERIN REDUCTASES

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The experimental and computational study of enzyme mechanisms has a long and illustrious history, but a detailed accounting of the molecular events remains incomplete for many important systems. Dihydrofolate reductase (DHFR) has been one of the most widely studied enzymes, but despite decades of research, neither atomistic nor electronic structure simulations are able to accurately rationalise the catalytic cycle. Recent simulation studies, using QM/MM and linear-scaling semi-empirical QM, point to polarisation effects as contributing significantly to stabilisation of the transition state in the hydride-transfer step of the reaction.

We present a work in progress investigating the electrostatics of the active sites of *E. coli* DHFR (normal cytosolic DHFR), *L. major* pteridine reductase (PTR1), and bacterial plasmid DHFR R67 (a so-called Type II DHFR). Although these three enzymes can catalyze the same reactions of reduction of the fully oxidized or 7,8-dihydro pterin moiety of folate or biopterin substrates using NADPH as the cofactor, they differ radically in primary sequence, tertiary and quaternary structure, and active-site architecture. Nonetheless, the disposition of certain key residues and crystallographically-resolved water molecules in the active sites appear to be similar among the three enzymes. As the catalytic competence and substrate specificity of these three enzymes differ, we hope to account for these differences with reference to the variation in active sites.

Crystallographic starting structures for the reactive ternary complexes are unavailable, so initial model construction for PTR1 and R67 involves docking of substrate and cofactor in the enzyme. We present preliminary results including details of model preparation, docking (using Autodock), model refinement and validation (using MD simulation and linear-scaling semi-empirical QM calculations) and comparative electrostatic maps.

ASB Poster Session 5PF.4 - Glass Foya - 1100-1140

DO MICROTUBULES ARCHITECTURE OBEY FULLERENIC PRINCIPLES?

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[1]Department of Physiology, The University of Sydney, NSW, Sydney 2006, Australia: [2]Department of Radiology, The University of Pennsylvania, PA, Philadelphia 19104, USA: [3]Department of Physics, The University of J.J. Strossmayer, Croatia, Osijek 31000: [4]Australian Institute of Nuclear Science and Engineering, NSW, Lucus Heights 2234, Australia: [5]Department of Pathology, The University of Sydney, NSW, Sydney 2006, Australia

Our investigations have shown that the ends of microtubules are often curled in the presence of calcium (Fig 1), and their intermediary part is multiple curved. In the absence of calcium, the microtubules are stiff straight cylinders with blunt (Fig 2) or tapered ends. To explain this phenomenon we propose that microtubule architecture is based on hexagonal and heptagonal motifs, found in fullerenic nanotubes as well as in some viruses [Ganser et al., Science (1999), 283, 80-83;S Martel et al., The Journal of Physical Chemistry (1999) 103(36) 7551-7556]. If, during assembly, tubulin dimmers form hexagonal motifs, then only straight and stiff microtubules will emerge. If, on the other hand, heptagonal motifs are formed, then negative curvature of protofilaments, are induced leading to abrupt microtubule depolymerization, or the growth may stop. In this paper we describe the most likely steps of the basic motif formation as well as subsequent microtubule formation. Microtubule destabilization in the presence of calcium is modeled using negative spontaneous curvature caused by the presence of anomalous tubulin.

Fig 1. Curled end of microtubule in the In the presence of 2mM calcium

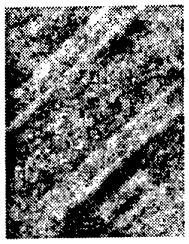

Fig 2. Blunt end of microtubule In calcium free solution

ASB Poster Session 5PF.4 - Glass Foya - 1100-1140

OPTOELECTRONIC AND STRUCTURAL STUDIES OF DIATOMS

J. M. Ferris[1], M. R. Phillips[2], C. J. Garvey[1], K. S. A. Butcher[3], and W. Vyverman[4]

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There is developing interest in the ability of biological systems to lay down inorganic matrices such as silica. Among the drivers of this research are silicon-based computing technologies and the move towards ever decreasing physical scales of technological devices. The micro-algae, diatoms, are amongst natures best architects of intricately shaped silica structures. Diatoms routinely assemble silica 'shells' at scales of tens to a few μm, which have regularly spaced pores to <10nm width. We have investigated the luminosity of several types of diatomaceous material, including field-collected benthic diatoms from shallow streams and culture-grown single species (eg Achnanthes subsessilis) as a means of probing the potential influence of the templated structure on the optoelectronic properties of the silica. Cathodoluminescence and photoluminescence data are presented. The porosity of this material is being characterised with an aim to correlating these results with the luminescence studies. Small angle neutron scattering (SANS) and ultra-small angle neutron scattering (USANS) measurements are being used to characterise bulk porosity over a range of length scales from angstroms to tens of microns. These results are being compared with two dimensional high resolution scanning electron micrographs obtained with a Schottky field emission gun scanning electron microscope.

SATURDAY September 20th

SCIENTIFIC PROGRAMME

Australian Society of Biophysics Meeting

University of Adelaide (exact venue to be notified to ASNB members on Friday 19th)

Plenary Lecture Session - 0930-1030

Chairperson: to be advised

6AU1 Robertson Award Lecture

Morning Tea

Invited Lecture Session - 1100-1230 - Radiation & Biological Tissues

Chairperson: to be advised

6BU1 Tim Van Doorn
PROTONS

6BU2 Derel Abbott
T RAYS

6BU3 Setayesh Behin-Ain
SINGLE PHOTON DETECTION IN TIME-RELATED TRANSILLUMINATION IMAGING

Lunch

Oral Paper Session 6CU - 1400-1500 - Reactivity

Chairperson: to be advised

6CU1 W Hillier
FTIR DIFFERENCE SPECTRA OF CYTOCHROME C OXIDASE REVEAL A COUPLING OF HISTIDINE IN THE REDUCTION CYCLE

6CU2 C Curtain
H2O2 PRODUCTION IS CENTRAL TO THE NEUROTOXICITY OF THE B-AMYLOID PEPTIDE FROM ALZHEIMER'S DISEASE; THE ROLE OF A TYROSINE FREE RADICAL IN ELECTRON TRANSFER

6CU3 WS Chow
ELECTRON FLUXES THROUGH PHOTOSYSTEM 1 IN CUCUMBER LEAF DISCS PROBED BY FAR-RED LIGHT

6CU4 JG Fernandez-Velasco
A NEW CATALYTIC SITE IN THE CYTOCHROME B_6 F COMPLEX OF CHLAMYDOMONAS REINHARDTII

Afternoon Tea

Oral Paper Session 6DU - 1530-1630

Chairperson: to be advised

6DU1 GP Jones
ANTHOCYANIN CHARGE EQUILIBRIA AND PKA'S BY ELECTROPHORESIS: A NEW VIEW OF THE COLOUR OF WINE AND ROSES

6DU2 JE Morrison
SURFACE PROPERTIES OF LEAVES MONITORED WITH THE NEW IMAGING-PAM

6DU3 HGL Coster
BILAYERS ON SILICON

6DU4 TC Chilcott
BIO-FUNCTIONALISING AND PASSIVATING SILICON

Close of ASB Meeting
ASB AGM - 1730
ASB Dinner - 1900

ASB Saturday Sessions - University of Adelaide - 1400-1415

FTIR DIFFERENCE SPECTRA OF CYTOCHROME *C* OXIDASE REVEAL A COUPLING OF HISTIDINE IN THE REDUCTION CYCLE

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The catalytic steps of O_2 reduction and pathways of proton pumping in the heme-Cu terminal oxidases present several fascinating mechanistic questions. In the work presented here we have isolated cytochrome c oxidase from *R. sphaeroides* and bovine heart, and used this for a series of electrochemically mediated FTIR difference spectroscopy measurements to examine the involvement of histidine in the catalytic cycle. Through both global ^{15}N labeling and specific ^{15}N histidine incorporation in the bacterial oxidase, a characteristic histidine vibration at 1104 cm^{-1} is found. This mode is ^{15}N sensitive, showing a 10 cm^{-1} downshift, and is also present in the mammalian oxidase enzyme. We will present measurements of simultaneous IR and UV-Vis optical spectra where we correlate the appearance of the histidine mode with the reduction status of hemes a/a_3. The spectroscopic finding of a FTIR active histidine residue coupling provides an indication for a functional role of histidine in the O_2 reduction chemistry and associated proton pumping sequence.

ASB Saturday Sessions - University of Adelaide - 1415-1430

H2O2 PRODUCTION IS CENTRAL TO THE NEUROTOXICITY OF THE B-AMYLOID PEPTIDE FROM ALZHEIMER'S DISEASE; THE ROLE OF A TYROSINE FREE RADICAL IN ELECTRON TRANSFER

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A form of beta-amyloid peptide (A_) ending at amino acid 42 is a major component of the amyloid plaques in Alzheimer's disease brains. A_ in the presence of Cu^{2+} and a reducing substrate catalyses the production of H_2O_2, which has been implicated in the neurotoxicity of these peptides_. The mechanism by which the A_ /Cu^{2+} produces H_2O_2 has not been explained, although it is known that it coordinates the redox active transition metals copper and iron to catalytically generate reactive oxygen species, probably via free radical formation on the peptide. Although such free radicals have been demonstrated by spin trapping, their significance is unclear and has been disputed because of impurities in spin traps used by some workers or because of the failure to take into account small traces of transition metals in the A_ preparations used. Using density functional theory calculations to define better the chemical mechanisms that drive the catalytic production of H_2O_2 by A_ /Cu^{2+} we have identified the tyrosine at position 10 as a key residue. The relative stability of tyrosyl radicals facilitates the electron transfers needed to drive the reaction. With the aid of a spin trap for carbon centered radicals (2-methyl-2-nitrosopropane) we could show the presence of a free radical on this residue in A_ /Cu^{2+} in the presence of a suitable reducing agent, confirming the theoretical result. Mutating the tyrosine residue to alanine inhibited H_2O_2 production, abolished neurotoxicity and almost eliminated the carbon centred radical, providing further confirmation of the importance of the tyrosine.

ASB Saturday Sessions - University of Adelaide - 1430-1445

ELECTRON FLUXES THROUGH PHOTOSYSTEM 1 IN CUCUMBER LEAF DISCS PROBED BY FAR-RED LIGHT

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Upon illumination, P700 (a special chlorophyll pair in Photosystem 1) is oxidised. While the electron is transferred onwards along a chain of carriers, the hole is filled by an electron ultimately originating from water molecules split in Photosystem 2 (also during illumination) or from elsewhere. Far-red light, predominantly absorbed by Photosystem 1, was used to photo-oxidise P700 to a steady-state, in which about 90% of P700 was in the form $P700^+$, monitored as an absorbance increase at 810 nm relative to 860 nm. On cessation of far-red illumination, $P700^+$ was re-reduced with multi-phasic kinetics, characterised by three exponential decays with rate coefficients $k_1 \sim 10\ s^{-1}$, $k_2 \sim 1\ s^{-1}$ and $k_3 \sim 0.1\ s^{-1}$. The initial rate of re-reduction of $P700^+$ at the instant of turning off far-red light, contributed by each phase, is given by the product of the rate coefficient and the amplitude for that phase; these rates are assumed equal to the electron fluxes to $P700^+$ at steady state. The three fluxes are interpreted as reflecting three pathways of electron flow to $P700^+$. Possible pathways of electron flow to $P700^+$ will be discussed.

ASB Saturday Sessions - University of Adelaide - 1445-1500

A NEW CATALYTIC SITE IN THE CYTOCHROME B_6 F COMPLEX OF *CHLAMYDOMONAS REINHARDTII*

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The Q-cycle model for the operation of the cytochrome (cyt) b_6f complex is based on the "oxidant-induced-reduction" phenomenon and proposes that the obligatory sequence of events that, in thylakoids after a flash, leads to the reduction of cyt *b* is: 1) oxidation of cyt *f* by plastocyanin (PC), 2) oxidation of Fe_2S_2 (Rieske) center by cyt *f* (i.e. re-reduction of cyt *f*), 3) oxidation of PQH_2 to semiquinone by Fe_2S_2 center at the Q_o site, and 4) reduction of the cyt *b* chain by semiquinone. Events 3 and 4 constitute a "concerted reaction". Therefore cyt *b*(s) should be reduced no faster than cyt *f* (1). Similarly, the re-reductions of PC and P700 that, after a train of flashes, are sensitive to inhibitors of the Qo site should be no faster than the re-reduction of cyt *f* (with a train of flashes the pools of in-the-dark reduced PC and cyt *f* are completely oxidized).

Those predictions are not verified. In wild type and/or cyt *f* mutants the Qo site-inhibitors-sensitive reductions of cyt *b*, PC, and P700 are unexpectedly fast, i.e. uncoupled from the reduction of cyt *f*, indicating that photosynthetic electron transfer can bypass cyt *f* (2). The same conclusion is obtained from the steady-state linear photosynthetic electron transfer in intact cells (2). Therefore, we define a fourth catalytic site in the cyt b_6f complex: a cyt-*f*-heme-independent PC reductase. We name this new site and path as "site R" and "path R", respectively, in order to identify it as "only-(R)ieske-center dependent". We distinguish it from "site F" and "path F", that are the cyt *f*:PC oxidoreductase site and route involving the cyt (*f*) heme. The upper limit for the t1/2 of "site R" is 700 μs. The Rieske protein, with a lumenal protruding domain bearing the Fe_2S_2 center (3), could be the extra docking site for PC and the alternative electron transfer point. The affinity of PC for site R would be 2-3 fold weaker than the same for site F. In the cyt *f* mutants, most of the electron transfer activity would take place through path R. Electron transfer through path R is also possible in the wt, as evidenced by the Qo site-sensitive P700 and PC reductions, which are 3-4 fold faster than the corresponding cyt *f* reduction. The functionality of site R in the wt can also be evidenced through the red cyt *b*/ox cyt *f* ratio after a very low intensity flash: a cyt *b*/cyt *f* ratio of 2.5 necessarily means that, in those conditions, there are cyt b_6f complexes where cyt *f* is not oxidized but cyt *b* is reduced anyhow (in prep.). The functional meaning of the coexistence of paths F and R and their steady-state operating ratios in the wt remain to be defined. Thus, although cyt *f* is redox-active, the precise role of this conserved protein needs clarification. Cyt *f* could be part of the proton path connecting Q_o with the thylakoid lumen (4). 1) Berry, EA et al. (2000) *Ann. Rev. Biochem.* **69**, 1005. 2) Fernández-Velasco, JG et al. (2001) *J. Biol. Chem.* **276**, 30598 . 3) Carrell, CJ et al. (1997) *Structure* **5**, 1613. 4) Martinez, SE et al (1996) *Protein Sci.* **5**, 1081. (*) JGFV is at Photobioenergetics Group- RSBS-ANU, javier.fernandez@rsbs.anu.edu.au

ASB Saturday Sessions - University of Adelaide - 1530-1545

ANTHOCYANIN CHARGE EQUILIBRIA AND PKA'S BY ELECTROPHORESIS: A NEW VIEW OF THE COLOUR OF WINE AND ROSES

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Paper electrophoresis has been used over the range (pH 1.2-10.4) to measure apparent pKa values for malvidin-3-*O*-glucoside (pKa_1 1.76 ± 0.07, pKa_2 5.36 ± 0.04 and pKa_3 8.39 ± 0.07) and malvidin-3-*O*-(6-*p*-coumaryl)glucoside (pKa_1 0.90 ± 0.07 and pKa_2 4.45 ± 0.03). As a non-spectrophotometric procedure, this charge-dependent electrophoretic mobility method provides independent information on the net charge and colour of anthocyanins in the pH range (pH 3.2-3.8) for red wines. In this pH range, the colour from malvidin-3-glucoside in red wines is only consistent with the uncharged quinonoidal base as a major component of the equilibria. The colour enhancement observed in young red wines can be attributed to the π–π stacking of this uncharged species and not the traditionally accepted cationic flavylium species. This has important ramifications in the study of anthocyanin-based pigments in red wines in that the chemistry of the quinonoidal form needs to be considered rather than that of the flavylium form. Similarly, in the pH range 5.5 – 7.0 the principle anthocyanin species of malvidin-3-glucoside is the mono anion; for acylated anthocyanins the mono anion predominates at even lower pH. This implies that in those flowers where colour is due to anthocyanins the chelation of metal ions to the quinonoidal mono anion rather than to carboxylic acid groups on copigment molecules present in the chromoplasts may dictate colour hue and stability.

ASB Saturday Sessions - University of Adelaide - 1545-1600

SURFACE PROPERTIES OF LEAVES MONITORED WITH THE NEW IMAGING-PAM

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A new fluorometer, the IMAGING-PAM, was investigated to assess its suitability for monitoring changes in leaf surface properties. Leaves of cauliflower (*Brassica oleracea* L.var. *botrytis)* were floated in a 0.1 mmol solution of DCMU and then changes in leaf PSII photochemistry were detected using the newly designed fluorometer. The effect on leaf surface integrity by an insecticidal agent, applied 4 days prior to the DCMU investigations, was measured. During the DCMU investigation leaves were exposed to a constant actinic irradiance (160 μmol m^{-2} s^{-1}) and saturating pulses (1800 μmol m^{-2} s^{-1}), applied at 60 second intervals. Effective PSII quantum yield was calculated according to the formula ($\Delta F/F_m'$) (Genty *et al.* 1989). Yield was imaged with a CCD camera and the data processed by the accompanying software (Walz GmbH, Effeltrich, Germany). Cauliflower leaves treated with the insecticidal agent showed a reduction in yield with time compared to untreated leaves, indicating that the insecticidal agent had compromised the structural integrity of the surface of the leaves. From our initial investigations it is apparent that the IMAGING-PAM is an extremely useful instrument for assessing leaf properties and, in particular, leaf surface integrity.

ASB Saturday Sessions - University of Adelaide - 1600-1615

BILAYERS ON SILICON

T. Böcking[1,2], T.C. Chilcott[1], H.G.L. Coster[1], K.D. Barrow[2], M. James[3]

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Planar lipid bilayers have been widely used as model systems to study transport processes and molecular interactions occurring at biological membranes including conduction through ion channels and the effect of pharmacologically important molecules such as anaesthetics and antibiotics on membrane structure. The instability of completely freestanding bilayer lipid membranes (BLM) of large and sometimes of highly variable area and thickness has led to the development of bilayers covalently anchored to a solid support. These highly stable systems rely on anchoring lipids of one leaflet of the bilayer to an oxide or gold surface. However, the electrical impedance of these bilayers was orders of magnitude less than that of the more biologically relevant freestanding variety. Further, the electrical impedance of the (Gouy-Chapman) ionic double layer that the gold substrates form with electrolytes is comparable with that of freestanding bilayers and therefore dominates measurements of the total impedance of anchored bilayer systems. Furthermore gold does not readily yield atomically flat surfaces nor does it facilitate X-ray and neutron based verifications of the attachment process. In contrast, atomically flat surface are readily obtained with silicon and doped silicon has a relative low dielectric constant that permits X-ray and neutron based analyses and reduces problems associated with the type of ionic double layers formed at the gold-electrolyte interface. We present X-ray reflectometry, X-ray photoelectric spectroscopy (XPS) and electrical impedance spectroscopy (EIS) characterisations of the chemical processes required to attach bilayers to atomically-flat silicon (111) surfaces. We have produced a hybrid bilayer with one lipid leaflet attached directly to the silicon surface. EIS characterisations of this system reveal that the impedance of the ionic double layer in the silicon-carbon system is orders of magnitude smaller than that of the ionic double layer in gold-carbon systems. This demonstrates that EIS characterisations of the type that yielded 0.1 nano-meter resolutions on freestanding bilayers are also possible on bilayers anchored to silicon. Future work will focus on refinement of the processes towards producing biologically relevant tethered bilayers.

ASB Saturday Sessions - University of Adelaide - 1615-1630

BIO-FUNCTIONALISING AND PASSIVATING SILICON

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Surface modification of silicon by covalent attachment of unsaturated molecules (alkenes) to Si-H surfaces via Si-C bonds is currently an area of intensive research because of its applications for passivating or bio-functionalising semiconductor devices. Many biosensor designs utilise self-assembling monolayers of functional thiols to attach organic molecules to gold surfaces. However, unlike silicon, gold does not readily yield atomically flat surfaces nor does it facilitate X-ray and neutron based verifications of the attachment process. Further, a significant operational impediment arises from the extraordinarily high electrical impedance of the (Gouy-Chapman) ionic double layer that gold forms with biological aqueous environments. We present electrical impedance spectroscopy (EIS), X-ray reflectometry and X-ray photoelectric spectroscopy characterisations (XPS) of organic monolayers attached to atomically-flat silicon (111) surface via Si-C bonds. XPS verified the composition of the monolayers and in particular the absence of SiO_2 contamination. X-ray reflectometry provided estimates of the monolayer thickness and density. EIS characterisations revealed the impedance of the ionic double layer in silicon systems was orders of magnitude smaller than that in gold systems. This permitted EIS characterisation of electro-mechanical properties of an undecanamine monolayer as well as the voltage-dependence of sub-structural layers of the depletion region in the silicon substrate at 0.1 nano-meter resolution. The results demonstrate accurate means of characterising the bio-functionalising/passivating properties of organic molecules on silicon for biosensor applications.

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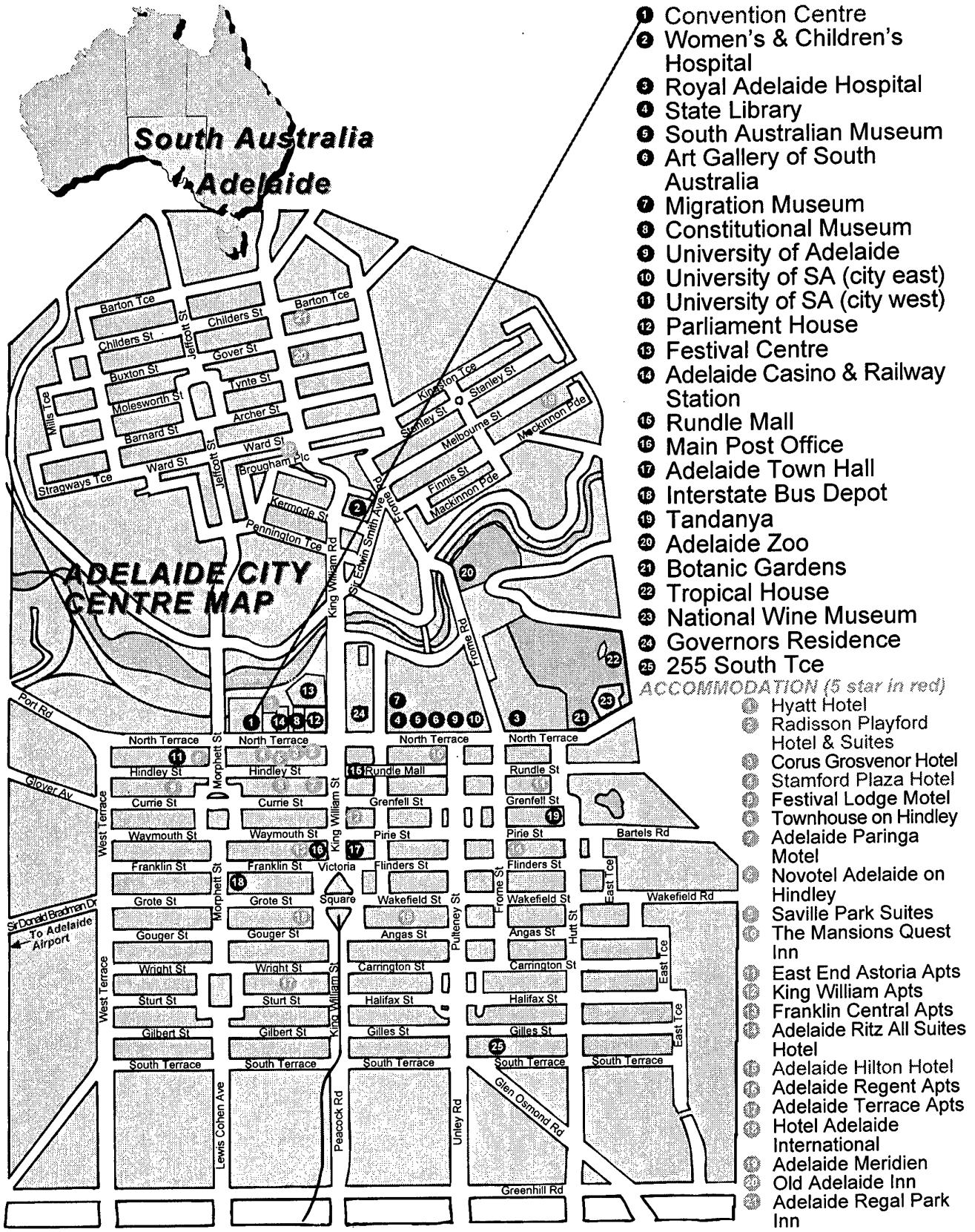
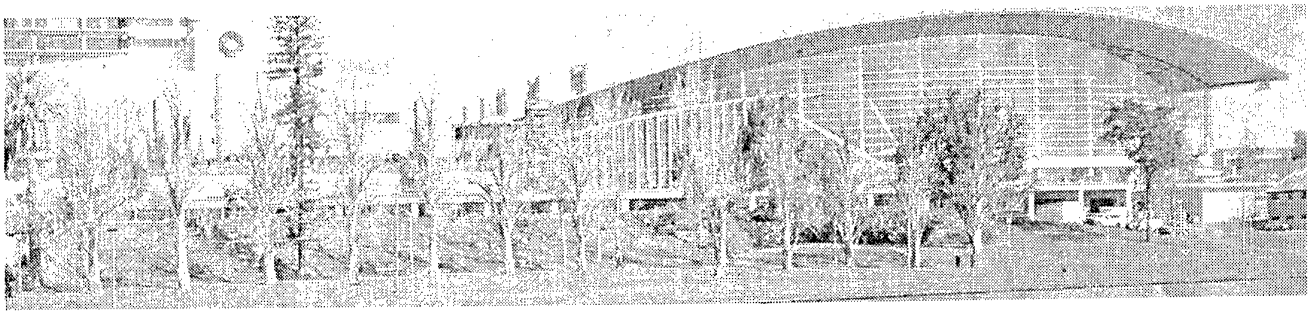


1. Convention Centre
2. Women's & Children's Hospital
3. Royal Adelaide Hospital
4. State Library
5. South Australian Museum
6. Art Gallery of South Australia
7. Migration Museum
8. Constitutional Museum
9. University of Adelaide
10. University of SA (city east)
11. University of SA (city west)
12. Parliament House
13. Festival Centre
14. Adelaide Casino & Railway Station
15. Rundle Mall
16. Main Post Office
17. Adelaide Town Hall
18. Interstate Bus Depot
19. Tandanya
20. Adelaide Zoo
21. Botanic Gardens
22. Tropical House
23. National Wine Museum
24. Governors Residence
25. 255 South Tce

ACCOMMODATION (5 star in red)

- Hyatt Hotel
- Radisson Playford Hotel & Suites
- Corus Grosvenor Hotel
- Stamford Plaza Hotel
- Festival Lodge Motel
- Townhouse on Hindley
- Adelaide Paringa Motel
- Novotel Adelaide on Hindley
- Saville Park Suites
- The Mansions Quest Inn
- East End Astoria Apts
- King William Apts
- Franklin Central Apts
- Adelaide Ritz All Suites Hotel
- Adelaide Hilton Hotel
- Adelaide Regent Apts
- Adelaide Terrace Apts
- Hotel Adelaide International
- Adelaide Meridien
- Old Adelaide Inn
- Adelaide Regal Park Inn



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